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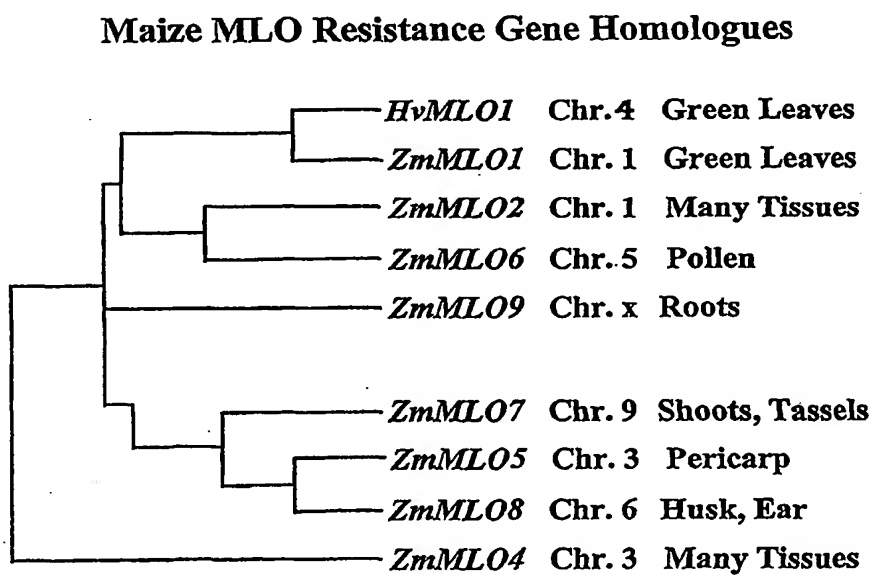

(57) Abstract

Compositions and methods for enhancing disease resistance in plants are provided. The method involves modulating the activity of *Mlo* sequences in the plant. Particular *Mlo* sequences are provided that can be manipulated to enhance pathogen resistance in modified plants. Transformed plants, plant cells, tissues, and seed are also provided having enhanced disease resistance.

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MANIPULATION OF *MLO* GENES TO ENHANCE DISEASE RESISTANCE IN PLANTS

FIELD OF THE INVENTION

The invention relates to the genetic manipulation of plants, particularly to transforming plants with genes that enhance disease resistance.

BACKGROUND OF THE INVENTION

Disease in plants is caused by biotic and abiotic causes. Biotic causes include fungi, viruses, bacteria, and nematodes. Of these, fungi are the most frequent causative agent of disease on plants. Abiotic causes of disease in plants include extremes of temperature, water, oxygen, soil pH, plus nutrient-element deficiencies and imbalances, excess heavy metals, and air pollution.

A host of cellular processes enables plants to defend themselves from disease caused by pathogenic agents. These processes apparently form an integrated set of resistance mechanisms that is activated by initial infection and then limits further spread of the invading pathogenic microorganism. This limitation of the pathogen intruder is frequently accomplished by localized containment of the intruder following a coordinated complex defense response.

Subsequent to recognition of a potentially pathogenic microbe, plants can activate an array of biochemical responses. Generally, the plant responds by inducing several local responses in the cells immediately surrounding the infection site. The most common resistance response observed in both nonhost and race-specific interactions is termed the "hypersensitive response" (HR). In the hypersensitive response, cells contacted by the pathogen, and often neighboring cells, rapidly collapse and dry in a necrotic fleck. Other responses include the deposition of callose, the physical thickening of cell walls by lignification, and the synthesis of various antibiotic small molecules and proteins. Genetic factors in both the host and the pathogen determine the specificity of these local responses, which can be very effective in limiting the spread of infection.

The hypersensitive response in many plant-pathogen interactions is specified by and dependent on the presence of two complementary genes, one from the host and one from the pathogen. These complementary genes are the resistance (*R*) gene in the plant and a corresponding avirulence (*avr*) gene in the pathogen. The interaction of the genes is associated with the rapid, localized cell death of the hypersensitive response. *R* genes that respond to specific bacterial, fungal, or viral pathogens, have been isolated from a variety of plant species and several appear to encode cytoplasmic proteins.

The resistance gene in the plant and the avirulence gene in the pathogen often conform to a gene-for-gene relationship. That is, resistance to a pathogen is only observed when the pathogen carries a specific avirulence gene and the plant carries a corresponding or complementing resistance gene. Because *avr-R* gene-for-gene relationships are observed in many plant-pathogen systems and are accompanied by a characteristic set of defense responses, a common molecular mechanism underlying *avr-R* gene mediated resistance has been postulated. A simple model which has been proposed is that pathogen *avr* genes directly or indirectly generate a specific molecular signal (ligand) that is recognized by cognate receptors encoded by plant *R* genes.

Both plant resistance genes and corresponding pathogen avirulence genes have been cloned. The plant kingdom contains thousands of *R* genes with specific specificities for viral, bacterial, fungal, or nematode pathogens. Although there are differences in the defense responses induced during different plant-pathogen interactions, some common themes are apparent among *R* gene-mediated defenses. The function of a given *R* gene is dependent on the genotype of the pathogen. Plant pathogens produce a diversity of potential signals, and in a fashion analogous to the production of antigens by mammalian pathogens, some of these signals are detectable by some plants.

The avirulence gene causes the pathogen to produce a signal that triggers a strong defense response in a plant with the appropriate *R* gene. However, expressing an avirulence gene does not stop the pathogen from being virulent on hosts that lack the corresponding *R* gene. A single plant can have many *R* genes, and a pathogen can have many *avr* genes.

Monogenic resistance mediated by recessive (*mlo*) alleles of the *Mlo* locus is different. It differs from race-specific incompatibility to single pathogen strains in that it is believed to confer a broad spectrum resistance to almost all known isolates of the fungal pathogen, and the resistance is apparently durable in the field despite extensive cultivation. Further, *mlo* resistance alleles have been obtained by mutagen treatment of susceptible wild-type *Mlo* varieties. These *mlo* plants exhibit a spontaneous leaf cell death phenotype under pathogen-free or even axenic conditions.

As noted, among the causative agents of infectious disease of crop plants, the phytopathogenic fungi play the dominant role. Phytopathogenic fungi cause devastating epidemics, as well as causing significant annual crop yield losses. All of the approximately 300,000 species of flowering plants are attacked by pathogenic fungi. However, a single plant species can be host to only a few fungal species, and similarly, most fungi usually have a limited host range.

Plant disease outbreaks have resulted in catastrophic crop failures that have triggered famines and caused major social change. Generally, the best strategy for plant disease control is to use resistant cultivars selected or developed by plant breeders for this purpose. However, the potential for serious crop disease epidemics persists today, as evidenced by outbreaks of the Victoria blight of oats and southern corn leaf blight.

Accordingly, molecular methods are needed to supplement traditional breeding methods to protect plants from pathogen attack. Particularly, methods are needed for broad spectrum resistance to pathogens.

SUMMARY OF THE INVENTION

Compositions and methods for creating or enhancing resistance to plant pests are provided. The method provides control of pathogens by modulating the expression of *Mlo* genes. Novel *Mlo* sequences are provided from maize. Such sequences can be utilized to modulate the expression of *Mlo* genes in plants, particularly maize, to enhance resistance to pathogens. Generally, such modulation will result in decreased or increased expression of native *Mlo* genes, preferably decreased expression. Such decreased expression can be effected by mutagenesis or expression of modified or antisense *Mlo* sequences described herein.

It is recognized that a variety of promoters will be useful in the invention the choice of which will depend in part upon the desired level of expression of the modified sequences in the plant or alternatively, in the plant organ. It is recognized that the levels of expression can be controlled to induce broad spectrum resistance resulting in levels of immunity in the plant or to induce cell death.

The methods of the invention find use in controlling plant pests, including fungal pathogens, viruses, nematodes, insects, and the like. Transformed plants and seeds, as well as methods for making such plants and seeds are additionally provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a sequence alignment for the maize *Mlo* homologues with the barley *Mlo* sequence. The barley sequence is designated as Hv*Mlo*1.

Figure 2 groups the *Mlo* sequences based on relatedness as well as the plant tissue from which it was isolated.

Figure 3 schematically illustrates a plasmid vector comprising the Zm*Mlo*1 antisense construct operably linked to the ubiquition promoter.

DETAILED DESCRIPTION OF THE INVENTION

Compositions of the invention include eight mutation-induced recessive alleles of maize set forth in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13 and 15. The maize sequences exhibit homology to the *Mlo* barley sequence (SEQ ID NO: 17). See, Büschges *et al.* (1997) *88*:695-705 and Tables 1 and 2. The isolated maize *Mlo* genes are involved in enhancing resistance to plant pests. In particular, the present invention provides for isolated nucleic acid molecules comprising nucleotide sequences encoding the amino acid sequences shown in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, or the nucleotide sequences encoding the DNA sequences deposited in a bacterial host with the American Type Culture Collection and assigned the Accession Nos. 98725, 98726, 98727, 98728, 98729 (SEQ ID NOS: 3, 5, 9, 11, 13 respectively). Further provided are polypeptides having an amino acid sequence encoded by a nucleic acid molecule described herein, for example those set forth in SEQ ID NOS: 1, 5, 3, 7, 9, 11, 13, and 15, those deposited with the American Type Culture Collection and assigned Accession Nos. 98725, 98726, 98727, 98728, 98729 (SEQ ID NOS: 3, 5, 9, 11, 13 respectively), and fragments and variants thereof.

Plasmids containing the nucleotide sequences of the invention were deposited with American Type Culture Collection, Manassas, Virginia, and assigned Accession Nos. 98725, 98726, 98727, 98728, 98729 (SEQ ID NOS: 3, 5, 9, 11, 13 respectively). These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. These deposits were made merely as a convenience for those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112.

The invention encompasses isolated or substantially purified nucleic acid or protein compositions. An "isolated" or "purified" nucleic acid molecule or protein, or biologically active portion thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. Preferably, an "isolated" nucleic acid is free of sequences (preferably protein encoding sequences) that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic

DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. A protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, 5%, (by dry weight) of contaminating protein. When the protein of the invention or biologically active portion thereof is recombinantly produced, preferably culture medium represents less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or non-protein-of-interest chemicals.

Fragments and variants of the disclosed nucleotide sequences and proteins encoded thereby are also encompassed by the present invention. By "fragment" is intended a portion of the nucleotide sequence or a portion of the amino acid sequence and hence protein encoded thereby. Fragments of a nucleotide sequence may encode protein fragments that retain the biological activity of the native protein and hence their altered expression enhances resistance to pathogens. Alternatively, fragments of a nucleotide sequence that are useful as hybridization probes generally do not encode protein fragments retaining biological activity. Thus, fragments of a nucleotide sequence may range from at least about 20 nucleotides, about 50 nucleotides, about 100 nucleotides, and up to the full-length nucleotide sequence encoding the proteins of the invention.

A fragment of a *Mlo* nucleotide sequence that encodes a biologically active portion of a Mlo protein of the invention will encode at least 15, 20, 25, 30, 40, 50, 75, 100, 200, 250, 300, 350, 400, 450, 500, or 550 contiguous amino acids, or up to the total number of amino acids present in a full-length MLO protein of the invention. (For example, 509, 499, and 492, amino acids for SEQ ID NOS: 6, 12, and 14 respectively.) Fragments of a *Mlo* nucleotide sequence that are useful as hybridization probes for PCR primers generally need not encode a biologically active portion of a MLO protein.

Thus, a fragment of a *Mlo* nucleotide sequence may encode a biologically active portion of a MLO protein, or it may be a fragment that can be used as a hybridization probe or PCR primer using methods disclosed below.

A biologically active portion of a MLO protein can be prepared by isolating a portion of one of the *Mlo* nucleotide sequences of the invention, expressing the encoded portion of the MLO protein (e.g., by recombinant expression *in vitro*), and assessing the activity of the encoded portion of the MLO protein. Nucleic acid molecules that are fragments of a *Mlo* nucleotide sequence comprise at least 16, 20, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, and 2,000 nucleotides, or up to the number of nucleotides present in a full-length *Mlo* nucleotide sequence disclosed herein.

By "variants" is intended substantially similar sequences. For nucleotide sequences, conservative variants include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence of one of the MLO polypeptides of the invention. Naturally occurring allelic variants such as these can be identified with the use of well-known molecular biology techniques, as, for example, with polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant nucleotide sequences also include synthetically derived nucleotide sequences, such as those generated, for example, by using site-directed mutagenesis but which still encode a MLO protein of the invention. Generally, nucleotide sequence variants of the invention will have at least 40%, 50%, 60%, 70%, generally, 80%, preferably 85%, 90%, up to 95%, 98% sequence identity to its respective native nucleotide sequence.

By "variant" protein is intended a protein derived from the native protein by deletion (so-called truncation) or addition of one or more amino acids to the N-terminal and/or C-terminal end of the native protein; deletion or addition of one or more amino acids at one or more sites in the native protein; or substitution of one or more amino acids at one or more sites in the native protein. Such variants may result from, for example, genetic polymorphism or from human manipulation.

The proteins of the invention may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of the Mlo proteins can be prepared by mutations in the DNA.

Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel (1985) *Proc. Natl. Acad. Sci. USA 82*:488-492; Kunkel *et al.* (1987) *Methods in Enzymol. 154*:367-382; US Patent No. 4,873,192; Walker and Gaastra, eds. (1983) *Techniques in Molecular Biology* (MacMillan Publishing Company, New York) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff *et al.* (1978) *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.), herein incorporated by reference. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be preferred.

Thus, the genes and nucleotide sequences of the invention include both the naturally occurring sequences as well as mutant forms. Likewise, the proteins of the invention encompass both naturally occurring proteins as well as variations and modified forms thereof. Such variants will continue to possess the desired ability to enhance resistance to pathogens when their expression is altered. Obviously, the mutations that will be made in the DNA encoding the variant must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See, EP Patent Application Publication No. 75,444.

The deletions, insertions, and substitutions of the protein sequences encompassed herein are not expected to produce radical changes in the characteristics of the protein. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays. That is, the activity can be evaluated by an enhanced resistance to pathogens when the expression of the protein sequences is altered.

Variant nucleotide sequences and proteins also encompass sequences and proteins derived from a mutagenic and recombinogenic procedure such as DNA shuffling. With such a procedure, one or more different *Mlo* coding sequences can be manipulated to create a new *Mlo* coding sequence possessing the desired properties. In this manner, libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides comprising

sequence regions that have substantial sequence identity and can be homologously recombined *in vitro* or *in vivo*. For example, using this approach, sequence motifs encoding a domain of interest may be shuffled between the *Mlo* gene of the invention and other known genes involved in pathogen resistance to obtain a new gene coding for a protein with an improved property of interest, such as an increased K_m in the case of an enzyme. Strategies for such DNA shuffling are known in the art. See, for example, Stemmer (1994) *Proc. Natl. Acad. Sci. USA 91*:10747-10751; Stemmer (1994) *Nature 370*:389-391; Crameri *et al.* (1997) *Nature Biotech. 15*:436-438; Moore *et al.* (1997) *J. Mol. Biol. 272*:336-347; Zhang *et al. (1997) Proc. Natl. Acad. Sci. USA 94*:4504-4509; Crameri *et al.* (1998) *Nature 391*:288-291; and U.S. Patent Nos. 5,605,793 and 5,837,458.

The maize *Mlo* homologues are associated with disease related loci as shown in Table 3. Mapping information of the sequences are given in Table 5. The maize homologue proteins are predicted to be membrane-anchored by at least six, and possibly seven membrane-spanning helices. The maize sequences find use in negative control function of the MLO protein in leaf cell death and in the onset of pathogen defense. Generally, the methods of the invention take advantage of the absence of MLO to prime responsiveness of the plant to disease.

The nucleotide sequences of the invention can be used to isolate corresponding sequences from other organisms, particularly other plants, more particularly other monocots. In this manner, methods such as PCR, hybridization, and the like can be used to identify such sequences based on their sequence homology to the sequences set forth herein. Sequences isolated based on their sequence identity to the entire *Mlo* sequences set forth herein or to fragments thereof are encompassed by the present invention.

In a PCR approach, oligonucleotide primers can be designed for use in PCR reactions to amplify corresponding DNA sequences from cDNA or genomic DNA extracted from any plant of interest. Methods for designing PCR primers and PCR cloning are generally known in the art and are disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York). See also Innis *et al.*,

eds. (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, New York); Innis and Gelfand, eds. (1995) *PCR Strategies* (Academic Press, New York); and Innis and Gelfand, eds. (1999) *PCR Methods Manual* (Academic Press, New York). Known methods of PCR include, but are not limited to, methods using paired primers, nested primers, single specific primers, degenerate primers, gene-specific primers, vector-specific primers, partially-mismatched primers, and the like.

In hybridization techniques, all or part of a known nucleotide sequence is used as a probe that selectively hybridizes to other corresponding nucleotide sequences present in a population of cloned genomic DNA fragments or cDNA fragments (*i.e.*, genomic or cDNA libraries) from a chosen organism. The hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ^{32}P, or any other detectable marker. Thus, for example, probes for hybridization can be made by labeling synthetic oligonucleotides based on the *Mlo* sequences of the invention. Methods for preparation of probes for hybridization and for construction of cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

For example, the entire sequence of the *Mlo* nucleotide sequences disclosed herein, or one or more portions thereof, may be used as a probe capable of specifically hybridizing to corresponding *Mlo* sequences and messenger RNAs. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique among *Mlo* sequences and are preferably at least about 10 nucleotides in length, and most preferably at least about 20 nucleotides in length. Such probes may be used to amplify corresponding *Mlo* sequences from a chosen plant by PCR. This technique may be used to isolate additional coding sequences from a desired plant or as a diagnostic assay to determine the presence of coding sequences in a plant. Hybridization techniques include hybridization screening of plated DNA libraries (either plaques or colonies; see, for example, Sambrook *et al.*

(1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

Hybridization of such sequences may be carried out under stringent conditions. By "stringent conditions" or "stringent hybridization conditions" is intended conditions under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, preferably less than 500 nucleotides in length.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C.

Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem. 138*:267-284: T_m = 81.5°C + 16.6 (log M) + 0.41 (%GC) - 0.61 (% form) - 500/L; where M is the

molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m, hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq$90% identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes,* Part I, Chapter 2 (Elsevier, New York); and Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

In general, sequences that encode for a Mlo protein and hybridize to the *Mlo* sequences disclosed herein will be at least 40% to 50% homologous, about 60% to 70% homologous, and even about 80%, 85%, 90%, 95% to 98% homologous or more with the disclosed sequences. That is, the sequence

similarity of sequences may range, sharing at least about 40% to 50%, about 60% to 70%, and even about 80%, 85%, 90%, 95% to 98% sequence similarity.

The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides: (a) "reference sequence", (b) "comparison window", (c) "sequence identity", (d) "percentage of sequence identity", and (e) "substantial identity".

(a) As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.

(b) As used herein, "comparison window" makes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith *et al.* (1981) *Adv. Appl. Math. 2*:482; by the homology alignment algorithm of Needleman *et al.* (1970) *J. Mol. Biol. 48*:443; by the search for similarity method of Pearson *et al.* (1988) *Proc. Natl. Acad. Sci. 85*:2444; by computerized implementations of these algorithms, including, but not limited to: CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, California; GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Drive, Madison, Wisconsin, USA; the CLUSTAL program is well described by Higgins *et al.* (1988) *Gene 73*:237-244 (1988); Higgins *et al.* (1989) *CABIOS 5*:151-153; Corpet *et al.* (1988) *Nucleic Acids Res. 16*:10881-90; Huang *et al.* (1992) *Computer Applications in the*

Biosciences 8:155-65, and Person *et al.* (1994) *Meth. Mol. Biol. 24*:307-331; preferred computer alignment methods also include the BLASTP, BLASTN, and BLASTX algorithms (see Altschul *et al.* (1990) *J. Mol. Biol. 215*:403-410). Alignment is also often performed by inspection and manual alignment.

(c) As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

(d) As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total

number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

(e)(i) The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least 70% sequence identity, preferably at least 80%, more preferably at least 90%, and most preferably at least 95%, compared to a reference sequence using one of the alignment programs described using standard parameters. One of skill in the art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning, and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least 60%, more preferably at least 70%, 80%, 90%, and most preferably at least 95%.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under stringent conditions. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. However, stringent conditions encompass temperatures in the range of about 1°C to about 20°C, depending upon the desired degree of stringency as otherwise qualified herein. Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides they encode are substantially identical. This may occur, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. One indication that two nucleic acid sequences are substantially identical is when the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

(e)(ii) The term "substantial identity" in the context of a peptide indicates that a peptide comprises a sequence with at least 70% sequence identity to a reference sequence, preferably 80%, more preferably 85%, most preferably at least 90% or 95% sequence identity to the reference sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm of Needleman *et al.* (1970) *J. Mol.*

Biol. 48:443. An indication that two peptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conservative substitution. Peptides that are "substantially similar" share sequences as noted above except that residue positions that are not identical may differ by conservative amino acid changes.

The invention is drawn to methods for creating or enhancing resistance in a plant to plant pests by modulating the activity of *Mlo* genes in the plant. While the invention is not bound by any particular mechanism of action, it is believed that the methods of the invention will result in broad-based resistance in the modified plant. Accordingly, the methods are also useful in protecting plants against fungal pathogens, viruses, nematodes, insects and the like.

By "modulating activity" is intended that the expression of the *Mlo* gene is altered in some manner. Such modulation (increase or decrease) of expression results in enhanced resistance to pathogens. Generally, the methods of the invention will result in a decrease in the native protein or in protein activity. Thus, plants and plant cells are obtained having altered levels of MLO protein, preferably a decrease in protein levels. Such plants, plant cells and plant tissues are "modified" in that MLO protein levels are altered. As noted below, various methods are available for creating modified plants, plant cells and plant tissues including transformation and transfection leading to altered *Mlo* expression in the modified plant, plant cell or tissue.

The invention is drawn to compositions and methods for inducing resistance in a plant to plant pests. Accordingly, the compositions and methods are also useful in protecting plants against fungal pathogens, viruses, nematodes, insects and the like.

By "disease resistance" is intended that the plants avoid the disease symptoms that are the outcome of plant-pathogen interactions. That is, pathogens are prevented from causing plant diseases and the associated disease symptoms, or alternatively, the disease symptoms caused by the pathogen is minimized or lessened. The methods of the invention can be utilized to protect

plants from disease, particularly those diseases that are caused by plant pathogens.

Pathogens of the invention include, but are not limited to, viruses or viroids, bacteria, insects, nematodes, fungi, and the like. Viruses include any plant virus, for example, tobacco or cucumber mosaic virus, ringspot virus, necrosis virus, maize dwarf mosaic virus, etc. Specific fungal and viral pathogens for the major crops include: Soybeans: *Phytophthora megasperma* fsp. *glycinea*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Diaporthe phaseolorum* var. *sojae* (*Phomopsis sojae*), *Diaporthe phaseolorum* var. *caulivora*, *Sclerotium rolfsii*, *Cercospora kikuchii*, *Cercospora sojina*, *Peronospora manshurica*, *Colletotrichum dematium* (*Colletotichum truncatum*), *Corynespora cassiicola*, *Septoria glycines*, *Phyllosticta sojicola*, *Alternaria alternata*, *Pseudomonas syringae* p.v. *glycinea*, *Xanthomonas campestris* p.v. *phaseoli*, *Microsphaera diffusa*, *Fusarium semitectum*, *Phialophora gregata*, Soybean mosaic virus, *Glomerella glycines*, Tobacco Ring spot virus, Tobacco Streak virus, *Phakopsora pachyrhizi*, *Pythium aphanidermatum*, *Pythium ultimum*, *Pythium debaryanum*, Tomato spotted wilt virus, *Heterodera glycines Fusarium solani*; Canola: *Albugo candida*, *Alternaria brassicae*, *Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Mycosphaerella brassiccola*, *Pythium ultimum*, *Peronospora parasitica*, *Fusarium roseum*, *Alternaria alternata*; Alfalfa: *Clavibater michiganese subsp. insidiosum*, *Pythium ultimum*, *Pythium irregulare*, *Pythium splendens*, *Pythium debaryanum*, *Pythium aphanidermatum*, *Phytophthora megasperma*, *Peronospora trifoliorum*, *Phoma medicaginis* var. *medicaginis*, *Cercospora medicaginis*, *Pseudopeziza medicaginis*, *Leptotrochila medicaginis*, *Fusarium*, *Xanthomonas campestris* p.v. *alfalfae*, *Aphanomyces euteiches*, *Stemphylium herbarum*, *Stemphylium alfalfae*; Wheat: *Pseudomonas syringae* p.v. *atrofaciens*, *Urocystis agropyri*, *Xanthomonas campestris* p.v. *translucens*, *Pseudomonas syringae* p.v. *syringae*, *Alternaria alternata*, *Cladosporium herbarum*, *Fusarium graminearum*, *Fusarium avenaceum*, *Fusarium culmorum*, *Ustilago tritici*, *Ascochyta tritici*, *Cephalosporium gramineum*, *Collotetrichum graminicola*, *Erysiphe graminis* f.sp. *tritici*, *Puccinia graminis* f.sp. *tritici*,

Puccinia recondita f.sp. *tritici*, *Puccinia striiformis*, *Pyrenophora tritici-repentis*, *Septoria nodorum*, *Septoria tritici*, *Septoria avenae*, *Pseudocercosporella herpotrichoides*, *Rhizoctonia solani*, *Rhizoctonia cerealis*, *Gaeumannomyces graminis* var. *tritici*, *Pythium aphanidermatum*, *Pythium arrhenomanes*, *Pythium ultimum*, *Bipolaris sorokiniana*, Barley Yellow Dwarf Virus, Brome Mosaic Virus, Soil Borne Wheat Mosaic Virus, Wheat Streak Mosaic Virus, Wheat Spindle Streak Virus, American Wheat Striate Virus, *Claviceps purpurea*, *Tilletia tritici*, *Tilletia laevis*, *Ustilago tritici*, *Tilletia indica*, *Rhizoctonia solani*, *Pythium arrhenomannes*, *Pythium gramicola*, *Pythium aphanidermatum*, High Plains Virus, European wheat striate virus; Sunflower: *Plasmophora halstedii*, *Sclerotinia sclerotiorum*, Aster Yellows, *Septoria helianthi*, *Phomopsis helianthi*, *Alternaria helianthi*, *Alternaria zinniae*, *Botrytis cinerea*, *Phoma macdonaldii*, *Macrophomina phaseolina*, *Erysiphe cichoracearum*, *Rhizopus oryzae*, *Rhizopus arrhizus*, *Rhizopus stolonifer*, *Puccinia helianthi*, *Verticillium dahliae*, *Erwinia carotovorum* pv. *carotovora*, *Cephalosporium acremonium*, *Phytophthora cryptogea*, *Albugo tragopogonis*; Corn: *Fusarium moniliforme* var. *subglutinans*, *Erwinia stewartii*, *Fusarium moniliforme*, *Gibberella zeae (Fusarium graminearum)*, *Stenocarpella maydi (Diplodia maydis)*, *Pythium irregulare*, *Pythium debaryanum*, *Pythium graminicola*, *Pythium splendens*, *Pythium ultimum*, *Pythium aphanidermatum*, *Aspergillus flavus*, *Bipolaris maydis* O, T *(Cochliobolus heterostrophus)*, *Helminthosporium carbonum* I, II & III *(Cochliobolus carbonum)*, *Exserohilum turcicum* I, II & III, *Helminthosporium pedicellatum*, *Physoderma maydis*, *Phyllosticta maydis*, *Kabatiella-maydis*, *Cercospora sorghi*, *Ustilago maydis*, *Puccinia sorghi*, *Puccinia polysora*, *Macrophomina phaseolina*, *Penicillium oxalicum*, *Nigrospora oryzae*, *Cladosporium herbarum*, *Curvularia lunata*, *Curvularia inaequalis*, *Curvularia pallescens*, *Clavibacter michiganense* subsp. *nebraskense*, *Trichoderma viride*, Maize Dwarf Mosaic Virus A & B, Wheat Streak Mosaic Virus, Maize Chlorotic Dwarf Virus, *Claviceps sorghi*, *Pseudonomas avenae*, *Erwinia chrysanthemi* pv. *zea*, *Erwinia carotovora*, *Corn stunt spiroplasma*, *Diplodia macrospora*, *Sclerophthora macrospora*, *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Peronosclerospora maydis*,

Peronosclerospora sacchari, *Sphacelotheca reiliana*, *Physopella zeae*, *Cephalosporium maydis*, *Cephalosporium acremonium*, Maize Chlorotic Mottle Virus, High Plains Virus, Maize Mosaic Virus, Maize Rayado Fino Virus, Maize Streak Virus, Maize Stripe Virus, Maize Rough Dwarf Virus; Sorghum: *Exserohilum turcicum*, *Colletotrichum graminicola (Glomerella graminicola)*, *Cercospora sorghi*, *Gloeocercospora sorghi*, *Ascochyta sorghina*, *Pseudomonas syringae* p.v. *syringae*, *Xanthomonas campestris* p.v. *holcicola*, *Pseudomonas andropogonis*, *Puccinia purpurea*, *Macrophomina phaseolina*, *Perconia circinata*, *Fusarium moniliforme*, *Alternaria alternata*, *Bipolaris sorghicola*, *Helminthosporium sorghicola*, *Curvularia lunata*, *Phoma insidiosa*, *Pseudomonas avenae (Pseudomonas alboprecipitans)*, *Ramulispora sorghi*, *Ramulispora sorghicola*, *Phyllachara sacchari*, *Sporisorium reilianum (Sphacelotheca reiliana)*, *Sphacelotheca cruenta*, *Sporisorium sorghi*, Sugarcane mosaic H, Maize Dwarf Mosaic Virus A & B, *Claviceps sorghi*, *Rhizoctonia solani*, *Acremonium strictum*, *Sclerophthona macrospora*, *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Sclerospora graminicola*, *Fusarium graminearum*, *Fusarium oxysporum*, *Pythium arrhenomanes*, *Pythium graminicola*, etc.

Nematodes include parasitic nematodes such as root-knot, cyst, lesion, and renniform nematodes, etc.

Insect pests include insects selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, Trichoptera, etc., particularly Coleoptera and Lepidoptera. Insect pests of the invention for the major crops include: Maize: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Helicoverpa zea*, corn earworm; *Spodoptera frugiperda*, fall armyworm; *Diatraea grandiosella*, southwestern corn borer; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Diatraea saccharalis*, surgarcane borer; *Diabrotica virgifera*, western corn rootworm; *Diabrotica longicornis barberi*, northern corn rootworm; *Diabrotica undecimpunctata howardi*, southern corn rootworm; *Melanotus spp.*, wireworms; *Cyclocephala borealis*, northern masked chafer (white grub); *Cyclocephala immaculata*, southern masked chafer (white grub); *Popillia japonica*, Japanese beetle;

Chaetocnema pulicaria, corn flea beetle; *Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*, corn leaf aphid; *Anuraphis maidiradicis*, corn root aphid; *Blissus leucopterus leucopterus*, chinch bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Hylemya platura*, seedcorn maggot; *Agromyza parvicornis*, corn blot leafminer; *Anaphothrips obscrurus*, grass thrips; *Solenopsis milesta*, thief ant; *Tetranychus urticae*, twospotted spider mite; Sorghum: *Chilo partellus*, sorghum borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Feltia subterranea*, granulate cutworm; *Phyllophaga crinita*, white grub; *Eleodes, Conoderus*, and *Aeolus spp.*, wireworms; *Oulema melanopus*, cereal leaf beetle; *Chaetocnema pulicaria*, corn flea beetle; *Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*; corn leaf aphid; *Sipha flava*, yellow sugarcane aphid; *Blissus leucopterus leucopterus*, chinch bug; *Contarinia sorghicola*, sorghum midge; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, twospotted spider mite; Wheat: *Pseudaletia unipunctata*, army worm; *Spodoptera frugiperda*, fall armyworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Agrotis orthogonia*, western cutworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Oulema melanopus*, cereal leaf beetle; *Hypera punctata*, clover leaf weevil; *Diabrotica undecimpunctata howardi*, southern corn rootworm; Russian wheat aphid; *Schizaphis graminum*, greenbug; *Macrosiphum avenae*, English grain aphid; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Mayetiola destructor*, Hessian fly; *Sitodiplosis mosellana*, wheat midge; *Meromyza americana*, wheat stem maggot; *Hylemya coarctata*, wheat bulb fly; *Frankliniella fusca*, tobacco thrips; *Cephus cinctus*, wheat stem sawfly; *Aceria tulipae*, wheat curl mite; Sunflower: *Suleima helianthana*, sunflower bud moth; *Homoeosoma electellum*, sunflower moth; *zygogramma exclamationis*, sunflower beetle; *Bothyrus gibbosus*, carrot beetle; *Neolasioptera murtfeldtiana*, sunflower seed midge; Cotton: *Heliothis virescens*, cotton budworm; *Helicoverpa zea*, cotton bollworm; *Spodoptera exigua*, beet armyworm; *Pectinophora gossypiella*, pink bollworm; *Anthonomus grandis grandis*, boll weevil; *Aphis gossypii*, cotton aphid; *Pseudatomoscelis*

seriatus, cotton fleahopper; *Trialeurodes abutilonea*, bandedwinged whitefly; *Lygus lineolaris*, tarnished plant bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Thrips tabaci*, onion thrips; *Franklinkiella fusca*, tobacco thrips; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, twospotted spider mite; Rice: *Diatraea saccharalis*, sugarcane borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Colaspis brunnea*, grape colaspis; *Lissorhoptrus oryzophilus*, rice water weevil; *Sitophilus oryzae*, rice weevil; *Nephotettix nigropictus*, rice leafhopper; *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug; Soybean: *Pseudoplusia includens*, soybean looper; *Anticarsia gemmatalis*, velvetbean caterpillar; *Plathypena scabra*, green cloverworm; *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Spodoptera exigua*, beet armyworm; *Heliothis virescens*, cotton budworm; *Helicoverpa zea*, cotton bollworm; *Epilachna varivestis*, Mexican bean beetle; *Myzus persicae*, green peach aphid; *Empoasca fabae*, potato leafhopper; *Acrosternum hilare*, green stink bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Hylemya platura*, seedcorn maggot; *Sericothrips variabilis*, soybean thrips; *Thrips tabaci*, onion thrips; *Tetranychus turkestani*, strawberry spider mite; *Tetranychus urticae*, twospotted spider mite; Barley: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Schizaphis graminum*, greenbug; *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug; *Euschistus servus*, brown stink bug; *Delia platura*, seedcorn maggot; *Mayetiola destructor*, Hessian fly; *Petrobia latens*, brown wheat mite; Oil Seed Rape: *Brevicoryne brassicae*, cabbage aphid; *Phyllotreta cruciferae*, Flea beetle; *Mamestra configurata*, Bertha armyworm; *Plutella xylostella*, Diamond-back moth; *Delia* ssp., Root maggots.

The present invention exploits the use of *Mlo* genes. The *Mlo* sequences provided herein can be utilized to alter the expression of native *Mlo* genes in plants. The *mlo* mutation confers recessive resistance to pathogens. Broad spectrum resistance in plants can be enhanced by defective *Mlo* genes.

While the invention is not bound by any model, *Mlo* could have a negative control function in leaf cell death. In this model, *Mlo* would suppress a

default cell suicide program in foliar tissue. Also, the MLO protein could have a specific negative regulatory function which works by down-regulating multiple disease-related functions. In this instance, spontaneous cell death in *mlo* mutant genotypes merely represents cell death because of accumulating activation of defense responses.

Several methods are available in the art for modulating the activity of *Mlo* genes. *Mlo* antisense sequences can be expressed in the plant cell. Such sequences will function to decrease expression of the maize *Mlo* genes as well as *Mlo* genes in other plants where the *Mlo* sequences share sequence identity.

It is recognized that with these nucleotide sequences, antisense constructions, complementary to at least a portion of the messenger RNA (mRNA) for the *Mlo* sequences can be constructed. Antisense nucleotides are constructed to hybridize with the corresponding mRNA. Modifications of the antisense sequences may be made as long as the sequences hybridize to and interfere with expression of the corresponding mRNA. In this manner, antisense constructions having 70%, preferably 80%, more preferably 85% sequence similarity to the corresponding antisensed sequences may be used. Furthermore, portions of the antisense nucleotides may be used to disrupt the expression of the target gene. Generally, sequences of at least 50 nucleotides, 100 nucleotides, 200 nucleotides, or greater may be used.

The antisense construct may be proximal to the 5'-terminus or capping site, downstream from the capping site, between the capping site and the initiation codon and may cover all or a portion of the non-coding region. Additionally, the sequence may bridge the non-coding and coding region, be complementary to all or part of the coding region, to the 3'-terminus of the coding region, or complementary to the 3'-untranslated region of the mRNA.

It is recognized that the particular site to which the antisense sequence binds and the length of the antisense sequence will vary depending upon the degree of inhibition desired, the uniqueness of the sequence, the stability of the antisense sequence, and the like. See, for example, U.S. Patent Nos. 5,453,566; 5,530,192; and 5,728,926; all of which are herein incorporated by reference.

The nucleotide sequences of the present invention may also be used in the sense orientation to suppress the expression of endogenous genes in plants.

Methods for suppressing gene expression in plants using nucleotide sequences in the sense orientation are known in the art. The methods generally involve transforming plants with a DNA construct comprising a promoter that drives expression in a plant operably linked to at least a portion of a nucleotide sequence that corresponds to the transcript of the endogenous gene. Typically, such a nucleotide sequence has substantial sequence identity to the sequence of the transcript of the endogenous gene, preferably greater than about 65% sequence identity, more preferably greater than about 85% sequence identity, most preferably greater than about 95% sequence identity. See, U.S. Patent Nos. 5,283,184 and 5,034,323; herein incorporated by reference.

Individual *Mlo* genes or sets of *Mlo* genes can be rendered nonfunctional by mutagenesis. Such mutagenesis techniques include transposon disruption and recovery of such disruptions by reverse genetics approaches. Likewise, transformation-mediated mutagenesis may be utilized.

Disruption may be accomplished by transformation with gene replacement or gene truncation-disruption. See, for example, Bowen *et al.* (1995) *Mol. Gen. Gent. 246*:196-205; Walz *et al.* (1993) *Curr. Gent. 25*:421-427; Sweigard *et al.* (1992) *Mol. Gen. Gent. 232*:183-190; Hohn and Desjardins (1992) *Mol. Plant Microbe Interact 5*:249-256; Weber and Laitner (1994) *Curr. Gent. 26*:461-467; Templeton *et al.* (1994) *Gene 142*:141-146; Gorlach *et al.* (1998) *Appl. Environ. Microbiol. 64*:385-391; Schaeffer *et al.* (1994) *Appl. Environ. Microbiol. 60*:594-598; and the like, herein incorporated by reference. See also, Kempin *et al.* (1997) *Nature 389*:802-803 and Koncz *et al.* (1992) *Plant Mol. Biol. 20*:963-976.

Alternatively, the naturally occurring *Mlo* sequence or sequences may be modified by site-directed mutagenesis. Such methods may be utilized to induce specific alterations in targeted genes. One means for site-directed mutagenesis includes targeting modification or mutation of the *Mlo* sequences by homologous recombination. The method involves the use of RNA-DNA hybrid oligonucleotides. Such nucleotides exploit the natural recombinogenicity of RNA-DNA hybrids. The oligonucleotides are duplex oligonucleotides that share homology with at least one *Mlo* sequence. While any region of the *Mlo* sequence can be targeted, it may be preferable to target

the 5' region of the *Mlo* sequence. See, for example, U.S. Patent No, 5,565,350 that describes chimeric oligonucleotides useful for targeted gene correction for use in cultured mammalian cells; as well as U.S. provisional application Serial No. 06/065,628 drawn to gene manipulation in plant cells, herein incorporated by reference. Such methods can be used to alter or disrupt the ATG start codon for the gene.

Alternatively, protein coding regions of *Mlo* genes can be altered in such a manner that the gene products or proteins perform their function in a dominant negative manner resulting in a resistant phenotype. See, for example Krylov *et al.* (1997) *Proc. Natl. Acad. Sci. USA 94*:12274-12279.

Where the method of the invention relies upon the expression of an altered *Mlo* sequence or an *Mlo* antisense sequence in a plant, a number of promoters can be used. The promoters can be selected from constitutive and/or inducible promoters. Such promoters include those from pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen; e.g., PR proteins, SAR proteins, beta-1,3-glucanase, chitinase, etc. See, for example, Redolfi *et al.* (1983) *Neth. J. Plant Pathol. 89*:245-254; Uknes *et al.* (1992) *The Plant Cell 4*:645-656; and Van Loon (1985) *Plant Mol. Virol. 4*:111-116. See, also copending application entitled "Inducible Maize promoters", filed and herein incorporated by reference.

Of interest are promoters that are expressed locally at or near the site of pathogen infection. See, for example, Marineau *et al.* (1987) *Plant Mol. Biol. 9*:335-342; Matton *et al.* (1989) *Molecular Plant-Microbe Interactions 2*:325-331; Somsisch *et al.* (1986) *Proc. Natl. Acad. Sci. USA 83*:2427-2430; Somsisch *et al.* (1988) *Molecular and General Genetics 2*:93-98; and Yang, Y (1996) *Proc. Natl. Acad. Sci. USA 93*:14972-14977. See also, Chen *et al.* (1996) *Plant J. 10*:955-966; Zhang and Sing (1994) *Proc. Natl. Acad. Sci. USA 91*:2507-2511; Warner *et al.* (1993) *Plant J. 3*:191-201; Siebertz *et al.* (1989) *Plant Cell 1*:961-968; and the references cited therein. Of particular interest is the inducible promoter for the maize PRms gene, whose expression is induced by the pathogen *Fusarium moniliforme* (see, for example, Cordero *et al.* (1992) *Physiological and Molecular Plant Pathology 41*:189-200).

Constitutive promoters include, for example, the core promoter of the Rsyn7 (copending application serial number 08/661,601), the 35S promoter, the core 35S promoter, and the like. Other constitutive promoters include, for example, U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142. See also, copending application entitled "Constitutive Maize Promoters" Provisional Application Serial No. 60/076,075 filed February 26, 1998, and herein incorporated by reference.

Tissue specific promoters include Yamamoto *et al.* (1997) *Plant J. 12(2)*:255-265; Kawamata *et al.* (1997) *Plant Cell Physiol. 38(7)*:792-803; Hansen *et al.* (1997) *Mol. Gen Genet. 254(3)*:337-343; Russell *et al.* (1997) *Transgenic Res. 6(2)*:157-168; Rinehart *et al.* (1996) *Plant Physiol. 112(3)*:1331-1341; Van Camp *et al.* (1996) *Plant Physiol. 112(2)*:525-535; Canevascini *et al.* (1996) *Plant Physiol. 112(2)*:513-524; Yamamoto *et al.* (1994) *Plant Cell Physiol. 35(5)*:773-778; Lam (1994) *Results Probl. Cell. Differ. 20*:181-196; Orozco *et al.* (1993) *Plant Mol. Biol. 23(6)*:1129-1138; Matsuoka *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A. 90(20)*:9586-9590; and Guevara-Garcia *et al.* (1993) *Plant J. 4(3)*:495-505.

The methods of the invention can be used with other methods for increasing pathogen resistance in plants. See, for example, Cai *et al.* (1997) *Science 275*:832-834; Roberts and Gallum (1984) *J. Heredity 75*:147-148; Ryerson and Heath (1996) *Plant Cell 8*:393-402 and Dangl *et al.* (1996) *Plant Cell 8*:1793-1807.

Altered *Mlo* sequences or antisense *Mlo* sequences of the invention can be introduced into any plant. The sequences to be introduced may be used in expression cassettes for expression in any plant of interest where expression in the plant cell is necessary. In other instances, such as for recombination, oligonucleotides are synthesized, purified and introduced into the plant cell.

Where expression cassettes are needed, such expression cassettes will comprise a transcriptional initiation region linked to the coding sequence or antisense sequence of the nucleotide of interest. Such an expression cassette is provided with a plurality of restriction sites for insertion of the sequence to be under the transcriptional regulation of the regulatory regions. The expression cassette may additionally contain selectable marker genes.

The transcriptional initiation region, the promoter, may be native or analogous or foreign or heterologous to the plant host. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. By foreign is intended that the transcriptional initiation region is not found in the native plant into which the transcriptional initiation region is introduced. As used herein a chimeric gene comprises a coding sequence operably linked to a transcription initiation region that is heterologous to the coding sequence.

While it may be preferable to express the sequences using heterologous promoters, the native promoter sequences may be used. Such constructs would change expression levels of a MLO protein in the plant or plant cell. Thus, the phenotype of the plant or plant cell is altered.

The transcriptional cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region, a DNA sequence of interest, and a transcriptional and translational termination region functional in plants. The termination region may be native with the transcriptional initiation region, may be native with the DNA sequence of interest, or may be derived from another source. Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also, Guerineau *et al.* (1991) *Mol. Gen. Genet. 262*:141-144; Proudfoot (1991) *Cell 64*:671-674; Sanfacon *et al.* (1991) *Genes Dev. 5*:141-149; Mogen *et al.* (1990) *Plant Cell. 2*:1261-1272; Munroe *et al.* (1990) *Gene 91*:151-158; Ballas *et al.* (1989) *Nucleic Acids Res. 17*:7891-7903; Joshi *et al.* (1987) *Nucleic Acid Res. 15*:9627-9639.

Nucleotide sequences of the invention are provided in expression cassettes for expression in the plant of interest. The cassette will include 5' and 3' regulatory sequences operably linked to the sequence of interest. The cassette may additionally contain at least one additional sequence to be cotransformed into the organism. Alternatively, the additional sequence(s) can be provided on another expression cassette.

Where appropriate, the gene(s) may be optimized for increased expression in the transformed plant. That is, the genes can be synthesized using plant-preferred codons for improved expression. See, for example, Campbell

and Gowri (1990) *Plant Physiol. 92*:1-11 for a discussion of host-preferred codon usage. Methods are available in the art for synthesizing plant-preferred genes. See, for example, U.S. Patent Nos. 5,380,831, 5,436,391, and Murray *et al.* (1989) *Nucleic Acids Res. 17*:477-498, herein incorporated by reference.

Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well-characterized sequences that may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. When possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures.

The expression cassettes may additionally contain 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein *et al.* (1989) *PNAS USA 86*:6126-6130); potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Allison *et al.* (1986); MDMV leader (Maize Dwarf Mosaic Virus); *Virology 154*:9-20), and human immunoglobulin heavy-chain binding protein (BiP), (Macejak *et al.* (1991) *Nature 353*:90-94); untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4) (Jobling *et al.* (1987) Nature *325*:622-625); tobacco mosaic virus leader (TMV) (Gallie *et al.* (1989) in Molecular Biology of RNA, ed. Cech (Liss, New York), pp. 237-256); and maize chlorotic mottle virus leader (MCMV) (Lommel *et al.* (1991) *Virology 81*:382-385). See also, Della-Cioppa *et al.* (1987) *Plant Physiol. 84*:965-968. Other methods known to enhance translation can also be utilized, for example, introns, and the like.

In preparing the expression cassette, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Towards this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, *in*

vitro mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g. transitions and transversions, may be involved.

The sequences of the present invention can be used to transform or transfect any plant. In this manner, genetically modified plants, plant cells, plant tissue, seed, and the like can be obtained. Transformation protocols as well as protocols for introducing nucleotide sequences into plants may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation. Suitable methods of introducing nucleotide sequences into plant cells and subsequent insertion into the plant genome include microinjection (Crossway *et al.* (1986) *Biotechniques 4*:320-334), electroporation (Riggs *et al.* (1986) *Proc. Natl. Acad. Sci. USA 83*:5602-5606, *Agrobacterium*-mediated transformation (Townsend *et al.*, U.S. Pat No. 5,563,055), direct gene transfer (Paszkowski *et al.* (1984) *EMBO J. 3*:2717-2722), and ballistic particle acceleration (see, for example, Sanford *et al.*, U.S. Patent No. 4,945,050; Tomes *et al.* (1995) "Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment," in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamborg and Phillips (Springer-Verlag, Berlin); and McCabe *et al.* (1988) *Biotechnology 6*:923-926). Also see Weissinger *et al.* (1988) *Ann. Rev. Genet. 22*:421-477; Sanford *et al.* (1987) *Particulate Science and Technology 5*:27-37 (onion); Christou *et al.* (1988) *Plant Physiol. 87*:671-674 (soybean); McCabe *et al.* (1988) *Bio/Technology 6*:923-926 (soybean); Finer and McMullen (1991) *In Vitro Cell Dev. Biol.* 27P:175-182 (soybean); Singh *et al.* (1998) *Theor. Appl. Genet. 96*:319-324 (soybean); Datta *et al.* (1990) *Biotechnology 8*:736-740 (rice); Klein *et al.* (1988) *Proc. Natl. Acad. Sci. USA 85*:4305-4309 (maize); Klein *et al.* (1988) *Biotechnology 6*:559-563 (maize); Tomes, U.S. Patent No. 5,240,855; Buising *et al.*, U.S. Patent Nos. 5,322,783 and 5,324,646; Tomes *et al. (1995)* "Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment," in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamborg (Springer-Verlag, Berlin) (maize); Klein *et al.* (1988) *Plant Physiol. 91*:440-444 (maize); Fromm *et al.* (1990) *Biotechnology 8*:833-839 (maize); Hooykaas-Van Slogteren *et al.* (1984) *Nature (London) 311*:763-764; Bowen *et al.*, U.S. Patent No. 5,736,369 (cereals); Bytebier *et al.* (1987) *Proc. Natl. Acad. Sci. USA*

84:5345-5349 (Liliaceae); De Wet *et al.* (1985) in *The Experimental Manipulation of Ovule Tissues,* ed. Chapman *et al.* (Longman, New York), pp. 197-209 (pollen); Kaeppler *et al.* (1990) *Plant Cell Reports 9*:415-418 and Kaeppler *et al.* (1992) *Theor. Appl. Genet. 84*:560-566 (whisker-mediated transformation); D'Halluin *et al.* (1992) *Plant Cell 4*:1495-1505 (electroporation); Li *et al.* (1993) *Plant Cell Reports 12*:250-255 and Christou and Ford (1995) *Annals of Botany 75*:407-413 (rice); Osjoda *et al.* (1996) *Nature Biotechnology 14*:745-750 (maize via *Agrobacterium tumefaciens*); all of which are herein incorporated by reference.

The modified plant may be grown into plants in accordance with conventional ways. See, for example, McCormick *et al.* (1986) *Plant Cell. Reports, 5*:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid having the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that the subject phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure the desired phenotype or other property has been achieved.

The *Mlo* sequences of the invention are also useful as molecular markers. Such markers are useful in breeding programs, particularly those aimed at improving disease resistance. The maize *Mlo* sequences have been mapped to chromosome locations and these positions related to known disease resistance loci.

The present invention provides a method of genotyping a plant comprising a polynucleotide of the present invention. Preferably, the plant is a monocot, such as maize or sorghum. Genotyping provides a means of distinguishing homologs of a chromosome pair and can be used to differentiate segregants in a plant population. Molecular marker methods can be used for phylogenetic studies, characterizing genetic relationships among crop varieties, identifying crosses or somatic hybrids, localizing chromosomal segments affecting monogenic traits, map based cloning, and the study of quantitative inheritance. See, e.g., *Plant Molecular Biology: A Laboratory Manual,* Chapter 7, Clark, Ed., Springer-Verlag, Berlin (1997). For molecular marker methods, see generally, The DNA Revolution by Andrew H. Paterson (1996)

(Chapter 2) in: Genome Mapping in Plants (ed. Andrew H. Paterson) by Academic Press/R. G. Landis Company, Austin, Texas, pp. 7-21.

The particular method of genotyping in the present invention may employ any number of molecular marker analytic techniques such as, but not limited to, restriction fragment length polymorphisms (RFLPs). RFLPs are the product of allelic differences between DNA restriction fragments caused by nucleotide sequence variability. As is well known to those of skill in the art, RFLPs are typically detected by extraction of genomic DNA and digestion with a restriction enzyme. Generally, the resulting fragments are separated according to size and hybridized with a probe; single copy probes are preferred. Restriction fragments from homologous chromosomes are revealed. Differences in fragment size among alleles represent an RFLP. Thus, the present invention further provides a means to follow segregation of a gene or nucleic acid of the present invention as well as chromosomal sequences genetically linked to these genes or nucleic acids using such techniques as RFLP analysis. Linked chromosomal sequences are within 50 centiMorgans (Cm), often within 40 or 30 Cm, preferably within 20 or 10 Cm, more preferably within 5, 3, 2, or 1 Cm of a gene.

In the present invention, the nucleic acid probes employed for molecular marker mapping of plant nuclear genomes selectively hybridize, under selective hybridization conditions, to a gene encoding a polynucleotide of the present invention. In preferred embodiments, the probes are selected from polynucleotides of the present invention. Typically, these probes are cDNA probes or *PstI* genomic clones. The length of the probes is discussed in greater detail, *supra*, but are typically at least 15 bases in length, more preferably at least 20, 25, 30, 35, 40, or 50 bases in length. Generally, however, the probes are less than about 1 kilobase in length. Preferably, the probes are single copy probes that hybridize to a unique locus in a haploid chromosome complement. Some exemplary restriction enzymes employed in RFLP mapping are EcoRI, EcoRv, and SstI. As used herein the term "restriction enzyme" includes reference to a composition that recognizes and, alone or in conjunction with another composition, cleaves at a specific nucleotide sequence.

The method of detecting an RFLP comprises the steps of (a) digesting genomic DNA of a plant with a restriction enzyme; (b) hybridizing a nucleic acid probe, under selective hybridization conditions, to a sequence of a polynucleotide of the present of said genomic DNA; (c) detecting therefrom a RFLP. Other methods of differentiating polymorphic (allelic) variants of polynucleotides of the present invention can be had by utilizing molecular marker techniques well known to those of skill in the art including such techniques as: 1) single-stranded conformation analysis (SSCP); 2) denaturing gradient gel electrophoresis (DGGE); 3) Rnase protection assays; 4) allele-specific oligonucleotides (ASOs); 5) the use of proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein; and 6) allele-specific PCR. Other approaches based on the detection of mismatches between the two complementary DNA strands include clamped denaturing gel electrophoresis (CDGE); heteroduplex analysis (HA); and chemical mismatch cleavage (CMC). Exemplary polymorphic variants are provided in Table I, *supra*. Thus, the present invention further provides a method of genotyping comprising the steps of contacting, under stringent hybridization conditions, a sample suspected of comprising a polynucleotide of the present invention with a nucleic acid probe. Generally, the sample is a plant sample; preferably, a sample suspected of comprising a maize polynucleotide of the present invention (e.g., gene, mRNA). The nucleic acid probe selectively hybridizes, under stringent conditions, to a subsequence of a polynucleotide of the present invention comprising a polymorphic marker. Selective hybridization of the nucleic acid probe to the polymorphic marker nucleic acid sequence yields a hybridization complex. Detection of the hybridization complex indicates the presence of that polymorphic marker in the sample. In preferred embodiments, the nucleic acid probe comprises a polynucleotide of the present invention.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Mlo Sequences

Eight maize *Mlo* homologue sequences have been identified. The nucleotide sequences (SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, and 15) and amino acid sequences (SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, and 16) are provided. DNA sequence analysis was performed according to Sambrook *et al.* (1989) *Mol. Cloning: A Laboratory Manual*, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, New York). The maize *Mlo* homologue/orthologue cDNA sequences were identified from mRNA isolated from various maize tissues and used to make cDNAs that are then cloned into vectors, usually pSPORT1. These cDNA libraries are EST sequenced via standard dye-fluorescence labeling and ABI machine electrophoresis and image capture. The *Mlo* homologues were identified by their blast score identity to the barley HvMLO1 published sequence. These maize clones, in particular the longest members of each contig or gene, were obtained and additional sequencing was done on them using oligonucleotide primers designed to internal portions of the cDNA and dye-fluorescence labeling and ABI machine electrophoresis and image capture. The complete edited sequences were assembled and analyzed.

The sequences from maize show sequence similarity to the published barley *Mlo* sequence as provided in Tables 1 and 2. Figure 1 provides a sequence alignment for the maize *Mlo* homologues with the barley *Mlo* sequence designated as Hv*Mlo*1. Figure 2 groups the *Mlo* sequences based on relatedness as well as the plant tissue from which it was isolated.

The ZmMLO genes were mapped by RFLP analysis using Southern blots of genomic DNA isolated from F2 and F3 and F4 segregating maize populations. The DNA was isolated using a modified CTAB adapted from the CERES RFLP Lab Manuel based on the Saghai-Mahoof procedure (Saghai-Mahoof *et al.* (1984) *Proc. Natl. Acad. Sci. USA 81*:8014-8018). Genomic DNA was cut with four restriction enzymes *BamH*I, *Eco*RI, *Eco*RV, and *Hind*III and run (5µg/lane) on 0.8% TAE agarose gels for 800 volt-hours. The gels were Southern blot transferred to Hybond-N membrane (Amersham Life Science), baked and UV-crosslinked. The ZmMLO cDNA inserts were liberated from its vector by cleaving with two restriction enzymes, usually *Sal*I

and *Not*I, and the insert was purified from agarose gels following electrophoresis. The clone was random prime labeled with ^{32}P-dCTP using the RTS (Gibco BRL) labeling kit. The cDNA inserts were used to probe parental screening blots to identify a mapping population with a RFLP polymorphism. Once a polymorphism was identified, the inserts were used to probe mapping blots containing DNA from segregating individuals. The map position was determined using MAPMAKER/EXP 3.0 (Lander *et al* (1987) *Genomics 1*:174-181) by scoring 86 segregating progeny as homozygous parent A, as homozygous parent B, or as heterozygous. The map position was assigned to an existing core RFLP map of either of the following three populations: ALEB9 (240 individuals) DRAG2 (283 individuals) or MARS (1075 individuals). Table 3 shows the association of maize *Mlo* homologues and disease related loci and QTLS.

TABLE 1

BestFit (Similarity/Identity)

Maize *MLO* Gene	HvMLO1	ZmMLO1	ZmMLO2	ZmMLO4	ZmMLO5	ZmMLO6	ZmMLO7	ZmMLO8	ZmMLO9
	Protein (Similarity/Identity) to:								
HvMLO1	100%	67	53	35	45	58	40	44	37
ZmMLO1	72	100%	45	35	38	51	39	43	39
ZmMLO2	61	56	100%	40	48	58	43	46	40
ZmMLO4	47	44	49	100%	46	38	39	40	46
ZmMLO5	54	47	56	56	100%	45	50	76	41
ZmMLO6	66	61	66	48	51	100%	41	43	39
ZmMLO7	48	49	52	48	58	52	100%	56	41
ZmMLO8	54	55	56	52	81	54	64	100%	43
ZmMLO9	48	48	49	51	56	50	51	56	100%

TABLE 2

Gap (Similarity/Identity)

Maize *MLO* Gene	Hv*MLO*1	Zm*MLO*1	Zm*MLO*2	Zm*MLO*4	Zm*MLO*5	Zm*MLO*6	Zm*MLO*7	Zm*MLO*8	Zm*MLO*9
				Protein Homology (Similarity/Identity) to:					
Hv*MLO*1	100%	66	43	31	33	55	36	40	32
Zm*MLO*1	71	100%	36	24	32	43	28	37	28
Zm*MLO*2	53	46	100%	36	36	57	39	45	34
Zm*MLO*4	42	31	46	100%	32	34	35	37	42
Zm*MLO*5	40	38	42	38	100%	43	42	60	36
Zm*MLO*6	63	51	64	43	49	100%	40	41	36
Zm*MLO*7	43	35	47	43	48	51	100%	51	40
Zm*MLO*8	49	48	54	49	67	52	59	100%	40
Zm*MLO*9	40	36	42	53	48	46	44	51	100%

TABLE 3

Association of Maize *MLO* Homologues and Disease Related Loci and QTLs

Maize *MLO* Homologue		Nearby Disease Resistance Loci or QTLs	
Zm*MLO*1	1.01	1.01	European Corn Borer QTL
		1.01/2	Northern Corn Leaf Blight QTL
Zm*MLO*2	1.04	1.03/6	Northern Corn Leaf Blight QTL
		1.04	Gray Leaf Spot QTL
		1.04	Maize Streak Virus (*msv*l)
		1.05	Stewart's Wilt QTL
Zm*MLO*4	3.05	3.04/5	European Corn Borer QTL
		3.04/5	Gibberella Stalk Rot QTL
Zm*MLO*5	3.06	3.07/8	Northern Corn Leaf Blight QTL
Zm*MLO*6a	5.05	5.04	Gibberella Stalk Rot QTL
		5.06	Northern Corn Leaf Blight QTL
Zm*MLO*6b	6.02/3	6.01	Southern Corn Leaf Blight (*rhm*1)
Zm*MLO*7	6.05/7		
Zm*MLO*8	9.04	9.05	Southwestern Corn Borer QTL
Zm*MLO*9	na		

TABLE 4

Mutator Insertion Mutants of Zm*MLO* Homologues (TUSC)

Zm*MLO2*

PV03 81D3	*C. heterostrophus* intermediate resistance (segregating as recessive)
PV03 81D2 (sib)	*C. heterostrophus* intermediate resistance (segregating as recessive)
PV03 108B1	*C. heterostrophus* intermediate resistance (segregating as recessive)
PV03 108A1 (sib)	*C. heterostrophus* resistance (segregating as recessive)
BT94 5F11	*C. heterostrophus* resistance (segregating as recessive); also leaf necrosis

The Trait Utility System for corn (TUSC) system was utilized to generate transposon mutants in the *Mlo* sequences in maize. The combination of transposon mutagenesis via *Mutator* and PCR-based selection of target-specific *Mu* insertions makes up the TUSC system. See, for example, Benson *et al.* (1995) *Plant Cell.* 7:75-84; Mena *et al.* (1996) *Science 274*:1537-1540; U.S. Patent Application No. 08/835,638 and U.S. Patent Application No. 08/262,056; all of which are herein incorporated by reference. Table 4 shows *Mutator* insertion mutants of Zm*Mlo* homologues.

Oligonucleotide primers based on *Mlo* maize sequences were designed and paired with *Mu* TIR primer in PCR reactions. The products of the screening process identify individuals in the collection having *Mu* elements inserted into *Mlo* sequences.

The gene-specific primers used for TUSC of ZmMLO2 are: 19005, 5'-AGTTATCCAAGGCATGCCGGTGGTAAAACT-3' (SEQ ID NO18); and 19007, 5'-AAGTCATCGAGGGAGGCATCACTTACAG-3' (SEQ ID NO:19). For TUSC the mutator-element specific primer used was: 9242, 5'-AGAGAAGCCAACGCCA(AT)CGCCTC(CT)ATTTCGTC-3' (SEQ ID NO:20). Seven positive "hits" to ZmMLO2 were identified. Twenty F2 seeds for each of the hits were planted in the greenhouse and screened as follows. Seeds were planted in trays, containing 2-inch wells for each seed, in Strong-Lite (circleR) Universal Mix potting soil (Universal Mix, Pine Bluff, Arkansas, U.S.A.) and grown in a greenhouse (16 hour day, 20.6-34.8°C, 50% average RH, 560-620 microEinsteins, of light both sun and halogen lamps, averaging 25 heat units per day. Plants were grown for 14-18 days until the V5 stage (ca. 25cm height) and inoculated with spores of *Cochliobolus heterostrophus* (Drechs.) Dreshs. Race 0, also known as *Bipolaris maydis* (Southern Leaf Blight) and *Cochliobolus carbonum* R. R Nelson, also known as *Bipolaris zeicola* (Helminthosporium Leaf Spot). For general leaf inoculation, spore suspensions of 4×10^4 per ml of 0.02% Tween-20 were sprayed as an aerosol on the leaves. Approximately 0.5 ml were applied per leaf late in the afternoon. The plants were then immediately covered with a plastic tent and kept at R.T. in order to enhance humidity and spore germination. The plastic tent was removed early in the morning, and the plants were returned to the greenhouse for the duration of the experiment, usually three days. Susceptibility to *Cochliobolus carbonum* was determined by either increased size or number of lesions. Resistance to *Cochliobolus*

heterostrophus was determined by either reduced size or number of lesions. Lesion mimics were also investigated; these mutations form spontaneous lesions in the absence of pathogen attach. Other phenotypes affecting growth and development were also noted.

TABLE 5

Maize *MLO* Gene	Zm*MLO*1	Zm*MLO*2	Zm*MLO*4	Zm*MLO*5	Zm*MLO*6a	Zm*MLO*6b	Zm*MLO*7	Zm*MLO*8	Zm*MLO*9
Gene Mapping Information									
Gene Complexity on Southern blot	1	1	1	1	2	2	1	1, 2	1, 2
Chromosome location (bin)	1.01	1.04	3.05	3.06	5.05	6.02/3	9.04	6.05/7	unassigned
Chromosome location (arm)	1S	1S	3L	3L	5L	6L	9L	6L	
Nearby Public Marker	BnI5.62	BnI12.06	umc10	bnI10.24	umc43	umc113	umc95	bnI5.47	
Distance from Public Marker	12.3 (down)	10.1 (down)	6.5 (down)	1.3 (up)	10.9 (down)	52.0 (up)	6.3 (down)	10.1 (up)	
Distance	15	5.9	35.2	1.3	1.8	52	10.7	10.1	
LOD Score	18.2	24.1	21.3	31.7	35.2	4.6	36.5	23.8	
RFLP Restriction Enzyme	EcoRI	HindIII	EcoRV	EcoRI	BamHI	BamHI	HindIII	EcoRV	
Mapping Population	ALEB9	ALEB9	DRAG2	ALEB9	DRAG2	DRAG2	DRAG2	DRAG2	DRAG2
Mapping Parents (Public Names)	R67/P38	R67/P38	17J/MWO	R67/P38	17J/MWO	17J/MWO	17J/MWO	17J/MWO	17J/MWO

TABLE 6

Maize *MLO* Gene	Hv*MLO*1	Zm*MLO* 1	Zm*MLO*2	Zm*MLO*4	Zm*MLO*5	Zm*MLO*6	Zm*MLO*7	Zm*MLO*8	Zm*MLO*9
Protein Structure Predictions									
Number of amino acids	533	224+	565	509	149+	515	499	492	469+
Calculated molecular weight	60,408		64,274	58,613		56,980	56,806	55,500	
Calculated pI	9.7		9.6	9.3		9.5	8.9	9.3	
Number of transmembrane domains	6 or 7		6 or 7	6 or 7		6 or 7	6 or 7	6 or 7	
Glycosylation sites? Asn-X-Ser/Thr	0	0	1	3	2	1	1	0	
Signal Peptide	No	No	No	No	No	No	No	No	No

Example 1: Transformation and Regeneration of Transgenic Plants

Immature maize embryos from greenhouse donor plants are bombarded with a plasmid containing the Zm*MLO*1 antisense sequence operably linked to the ubiquitin promoter (Figure 3). This plasmid also contains the selectable marker gene PAT (Wohlleben *et al.* (1988) *Gene* 70:25-37) that confers resistance to the herbicide Bialophos. Transformation is performed as follows. All media recipes are in the Appendix.

Preparation of Target Tissue

The ears are surface sterilized in 30% Chlorox bleach plus 0.5% Micro detergent for 20 minutes, and rinsed two times with sterile water. The immature embryos are excised and placed embryo axis side down (scutellum side up), 25 embryos per plate, on 560Y medium for 4 hours and then aligned within the 2.5-cm target zone in preparation for bombardment.

Preparation of DNA

A plasmid vector comprising the PAT selectable marker and the *ZmMlo1* antisense sequence operably linked to a the ubiquitin is made and precipitated onto 1.1 μm (average diameter) tungsten pellets using a $CaCl_2$ precipitation procedure as follows:

100 μl prepared tungsten particles in water
10 μl (1 μg) DNA in TrisEDTA buffer (1 μg total)
100 μl 2.5 M $CaCl_2$
10 μl 0.1 M spermidine

Each reagent is added sequentially to the tungsten particle suspension, while maintained on the multitube vortexer. The final mixture is sonicated briefly and allowed to incubate under constant vortexing for 10 minutes. After the precipitation period, the tubes are centrifuged briefly, liquid removed, washed with 500 ml 100% ethanol, and centrifuged for 30 seconds. Again the liquid is removed, and 105 μl 100% ethanol is added to the final tungsten particle pellet. For particle gun bombardment, the

tungsten/DNA particles are briefly sonicated and 10 µl spotted onto the center of each macrocarrier and allowed to dry about 2 minutes before bombardment.

Particle Gun Treatment

The sample plates are bombarded at level #4 in particle gun #HE34-1 or #HE34-2. All samples receive a single shot at 650 PSI, with a total of ten aliquots taken from each tube of prepared particles/DNA.

Subsequent Treatment

Following bombardment, the embryos are kept on 560Y medium for 2 days, then transferred to 560R selection medium containing 3 mg/liter Bialaphos, and subcultured every 2 weeks. After approximately 10 weeks of selection, selection-resistant callus clones are transferred to 288J medium to initiate plant regeneration. Following somatic embryo maturation (2-4 weeks), well-developed somatic embryos are transferred to medium for germination and transferred to the lighted culture room. Approximately 7-10 days later, developing plantlets are transferred to 272V hormone-free medium in tubes for 7-10 days until plantlets are well established. Plants are then transferred to inserts in flats (equivalent to 2.5" pot) containing potting soil and grown for 1 week in a growth chamber, subsequently grown an additional 1-2 weeks in the greenhouse, then transferred to classic 600 pots (1.6 gallon) and grown to maturity. Plants are monitored and scored for enhanced disease resistance as a result of antisense disruption of Zm*Mlo*1 function.

APPENDIX

272 V

Ingredient	Amount	Unit
D-I H_2O	950.000	Ml
MS Salts (GIBCO 11117-074)	4.300	G
Myo-Inositol	0.100	G
MS Vitamins Stock Solution ##	5.000	Ml
Sucrose	40.000	G
Bacto-Agar @	6.000	G

Directions:

@ = Add after bringing up to volume

Dissolve ingredients in polished D-I H_2O in sequence

Adjust to pH 5.6

Bring up to volume with polished D-I H_2O after adjusting pH

Sterilize and cool to 60°C.

= Dissolve 0.100 g of Nicotinic Acid; 0.020 g of Thiamine.HCL; 0.100 g of Pyridoxine.HCL; and 0.400 g of Glycine in 875.00 ml of polished D-I H_2O in sequence. Bring up to volume with polished D-I H_2O. Make in 400 ml portions. Thiamine.HCL & Pyridoxine.HCL are in Dark Desiccator. Store for one month, unless contamination or precipitation occurs, then make fresh stock.

Total Volume (L) = 1.00

288 J

Ingredient	Amount	Unit
D-I H_2O	950.000	Ml
MS Salts	4.300	g
Myo-Inositol	0.100	g
MS Vitamins Stock Solution ##	5.000	ml
Zeatin .5mg/ml	1.000	ml
Sucrose	60.000	g
Gelrite @	3.000	g
Indoleacetic Acid 0.5 mg/ml #	2.000	ml
0.1mM Abscisic Acid	1.000	ml
Bialaphos 1mg/ml #	3.000	ml

Directions:

@ = Add after bringing up to volume

Dissolve ingredients in polished D-I H_2O in sequence

Adjust to pH 5.6

Bring up to volume with polished D-I H_2O after adjusting pH

Sterilize and cool to 60°C.

Add 3.5g/L of Gelrite for cell biology.

= Dissolve 0.100 g of Nicotinic Acid; 0.020 g of Thiamine.HCL; 0.100 g of Pyridoxine.HCL; and 0.400 g of Glycine in 875.00 ml of polished D-I H_2O in sequence. Bring up to volume with polished D-I H_2O. Make in 400 ml portions. Thiamine.HCL & Pyridoxine.HCL are in Dark Desiccator. Store for one month, unless contamination or precipitation occurs, then make fresh stock.

Total Volume (L) = 1.00

560 R

Ingredient	Amount	Unit
D-I Water, Filtered	950.000	ml
CHU (N6) Basal Salts (SIGMA C-1416)	4.000	g
Eriksson's Vitamin Mix (1000X SIGMA-1511	1.000	ml
Thiamine.HCL 0.4mg/ml	1.250	ml
Sucrose	30.000	g
2, 4-D 0.5mg/ml	4.000	ml
Gelrite @	3.000	g
Silver Nitrate 2mg/ml #	0.425	ml
Bialaphos 1mg/ml #	3.000	ml

Directions:

@ = Add after bringing up to volume

= Add after sterilizing and cooling to temp.

Dissolve ingredients in D-I H_2O in sequence

Adjust to pH 5.8 with KOH

Bring up to volume with D-I H_2O

Sterilize and cool to room temp.

Total Volume (L) = 1.00

560 Y

Ingredient	Amount	Unit
D-I Water, Filtered	950.000	ml
CHU (N6) Basal Salts (SIGMA C-1416)	4.000	g
Eriksson's Vitamin Mix (1000X SIGMA-1511	1.000	ml
Thiamine.HCL 0.4mg/ml	1.250	ml
Sucrose	120.000	g
2,4-D 0.5mg/ml	2.000	ml
L-Proline	2.880	g
Gelrite @	2.000	g
Silver Nitrate 2mg/ml #	4.250	ml

Directions:

@ = Add after bringing up to volume

= Add after sterilizing and cooling to temp.

Dissolve ingredients in D-I H_2O in sequence

Adjust to pH 5.8 with KOH

Bring up to volume with D-I H_2O

Sterilize and cool to room temp.

** Autoclave less time because of increased sucrose**

Total Volume (L) = 1.00

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 5, lines 11-20

B. **IDENTIFICATION OF DEPOSIT** Further deposits are identified on an additional sheet ☐

Name of depository institution
American Type Culture Collection

Address of depositary institution *(including postal code and country)*
Manassa, Virginia USA

Date of deposit	Accession Numbers
	98725-98729

C. **ADDITIONAL INDICATIONS** *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. **DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** *(if the indicators are not for all designated States)*

E. **SEPARATE FURNISHING OF INDICATIONS** *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

Date of Deposit

For receiving Office use only

☑ This sheet was received with the international application

Authorized officer

For International Bureau use only

☐ This sheet was received with the International Bureau on:

Authorized officer

WHAT IS CLAIMED IS:

1. A method for creating or enhancing disease resistance in a plant, said method comprising modulating the expression of an *Mlo* nucleotide sequence wherein said nucleotide sequence is selected from the group consisting of:

(a) a nucleotide sequence encoding a maize *Mlo* gene;

(b) a nucleotide sequence encoding at least one of the maize MLO amino acid sequences selected from the group consisting of the sequences set forth in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, and 16;

(c) a nucleotide sequence comprising at least one of the nucleotide sequences set forth in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, and 15;

(d) a nucleotide sequence selected from the group consisting of sequences deposited in the American Type Culture Collection as Accession Nos. 98725, 98726, 98727, 98728, and 98729;

(e) a nucleotide sequence that encodes an antisense sequence corresponding to a sequence of a), b), c) or d);

(f) a nucleotide sequence which hybridizes under stringent conditions to one of the nucleotide sequences set forth in a), b), c), d) or e).

2. The method of claim 1, wherein said modulation comprises decreasing the expression of the *Mlo* sequence.

3. The method of claim 1, wherein said plant is a monocot.

4. The method of claim 3, wherein said monocot is maize.

5. A modified plant having altered levels of an MLO protein wherein said plant has stably incorporated into its genome a DNA construct, wherein said DNA construct comprises a nucleotide sequence operably linked

to a promoter capable of regulating transcription of said sequence in said plant, wherein said nucleotide sequence is selected from the group consisting of:

(a) a nucleotide sequence encoding a maize *Mlo* gene;

(b) a nucleotide sequence encoding at least one of the maize MLO amino acid sequences selected from the group consisting of the sequences set forth in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, and 16;

(c) a nucleotide sequence comprising at least one of the nucleotide sequences set forth in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, and 15;

(d) a nucleotide sequence selected from the group consisting of sequences deposited in the American Type Culture Collection as Accession Nos. 98725, 98726, 98727, 98728, and 98729;

(e) a nucleotide sequence that encodes an antisense sequence corresponding to a sequence of a), b), c) or d);

(f) a nucleotide sequence which hybridizes under stringent conditions to one of the nucleotide sequences set forth in a), b), c), d) or e).

6. The modified plant of claim 5, wherein said plant exhibits a decrease in the expression of a *Mlo* gene.

7. The modified plant of claim 5, wherein said plant is a monocot.

8. The modified plant of claim 7, wherein said monocot is maize.

9. The maize plant of claim 8, wherein said plant has decreased levels of the *Mlo* protein.

10. The maize plant of claim 8, wherein said maize plant comprises an insertion sequence in at least one maize *Mlo* gene.

11. Seed of the plant of any one of claims 5, 6, 7, 8, 9, and 10.

12. An isolated MLO protein, wherein said protein comprises an amino acid sequence selected from the group consisting of the MLO amino acid sequences set forth in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, and 16.

13. An isolated nucleotide sequence said sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a maize *Mlo* gene;

(b) a nucleotide sequence encoding at least one of the maize MLO amino acid sequences selected from the group consisting of the sequences set forth in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, and 16;

(c) a nucleotide sequence comprising at least one of the nucleotide sequences set forth in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, and 15;

(d) a nucleotide sequence selected from the group consisting of sequences deposited in the American Type Culture Collection as Accession Nos. 98725, 98726, 98727, 98728, and 98729;

(e) a nucleotide sequence that encodes an antisense sequence corresponding to a sequence of a), b), c) or d);

(f) a nucleotide sequence which hybridizes under stringent conditions to one of the nucleotide sequences set forth in a), b), c), d) or e).

14. A modified plant cell having an altered level of an MLO protein wherein said plant cell has stably incorporated into its genome a DNA construct, wherein said DNA construct comprises a nucleotide sequence operably linked to a promoter capable of regulating transcription of said sequence in said plant cell, wherein said nucleotide sequence is selected from the group consisting of :

(a) a nucleotide sequence encoding a maize *Mlo* gene;

(b) a nucleotide sequence encoding at least one of the maize MLO amino acid sequences selected from the group consisting of the sequences set forth in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, and 16;

(c) a nucleotide sequence comprising at least one of the nucleotide sequences set forth in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, and 15;

(d) a nucleotide sequence selected from the group consisting of sequences deposited in the American Type Culture Collection as Accession Nos. 98725, 98726, 98727, 98728, and 98729;

(e) a nucleotide sequence that encodes an antisense sequence corresponding to a sequence of a), b), c) or d);

(f) a nucleotide sequence which hybridizes under stringent conditions to one of the nucleotide sequences set forth in a), b), c), d) or e).

15. The modified plant cell of claim 14, wherein said cell exhibits a decrease in the expression of an *Mlo* gene.

16. The modified plant cell of claim 14, wherein said cell is from a monocot.

17. The modified plant cell of claim 16, wherein said monocot is maize.

CLUSTAL W (1.7) Multiple Sequence Alignments of HvMLO1 and 9 ZmMLO Homologues

```
HvMLO1  -MSDKKGVPARELPETPSWAVAVVFAAMVLVSVLMEHGLHKLGHWFQHRHKKALWEALEK
ZmMLO1  ----------------------------------------------------------
ZmMLO2  MGGDG----TRALDQTPTWAVAAVCAVIVAASILLEGFLHHLGQLLNKKRKKALFDALEK
ZmMLO6  MGGGGGGGNSRELDDTPTWAVASVCGVIVLISILLEKGLHHVGEFFSHRKKKAMVEALEK

ZmMLO7  ------MGKEATLAFTPTWVVAIVCLVIVSISLAAERSLHYLGKYLECKKQKALFSALQR
ZmMLO5  ----------------------------------------------------------
ZmMLO8  ---MAAEGEAAALEFTPTWIVAAVCSLIVLLSLVAERCLHYLGKTLKRKNQKPLFEALLK
ZmMLO9  ----------------------------------------------------------
ZmMLO4  ----MAAEQGRSLAETPTWSVATVTTLMVAACFLVERSLSRFAKWLRKTKRKAMLAALEK
Regions [---N-Terminus---] [---TM Helix 1---] [---Intervening Region 1-

HvMLO1  MKAELMLVGFISLLLIVTQDPIIAKICISED-AADVMWPCKRGTEGRKPS----------
ZmMLO1  ----------------------------------------------------------
ZmMLO2  VKSELMTLGFISLLLTVTGR-YIARICIPEG-AANTMLPCRLSGHSVAEEPKGHGRR---
ZmMLO6  VKAELMVLGFISLLLVFGQN-YIIKVCISNH-AANTMLPCKLEAAAVEGK-DGHGKEAAA

ZmMLO7  LKEELMLLGFISFVLSLSQG-FIVSICIPET-STDFMLPCNR-----GNS----------
ZmMLO5  ----------------------------------------------------------
ZmMLO8  VKEELMLLGFISLLLTVFQG-MIRRTCIPER-WTFHMLPCEKPDEKAGEAATMEHFVGTL
ZmMLO9  ----------------------------------------------------------
ZmMLO4  IREELMLLGVISLLLSQTAR-FISEICVPSSLFTSRFYICSESDYQDLLRNTDANQTALD
        ---] [------TM Helix 2-------] [-----Intervening Region 2-----

HvMLO1  ----------------------------------------K------YVDYCPEGKVA
ZmMLO1  ----------------------------------------------------------
ZmMLO2  ----------------------HLS----------------EDPTNLFSCRKGMVS
ZmMLO6  VVAGKKKVAVAVPGKKKKKAAAAADHLGGVVDWPPPYYAHNARMLAEASMATKCPEGKVP

ZmMLO7  ----------------------------------------RVAEEGAKICNKKGDVP
ZmMLO5  ----------------------------------------------------------
ZmMLO8  GRIGRR--------------------------------LLQEGTAGAEQCQKKGKVP
ZmMLO9  ------------------------------HSPRRNLKTSYHNHEGCREGYES
ZmMLO4  ----------------------------------KNMFGGQ-RLHVCGEGHEP
        ----------------------Intervening Region 2------------------

HvMLO1  LMSTGSLHQLHVFIFVLAVFHVTYSVITIALSRLKMRTWKKWETETTSLEYQFANDPAR-
ZmMLO1  ----------------------------------------------------------
ZmMLO2  LVSADGMHQLHIFVFFLAVFHVTFSFFTMSLGRAKTRIWKVWEKETCSPQYNYLNDPSK-
ZmMLO6  LISINALHQLHIFIFFLAVFHVSYSAITMALGRAKIRAWKEWEKEAAGQDYEFSHDPTR-

ZmMLO7  LLSLEALHQLHIFIFVLGLVHVVFCATTISFSGAKMRKWKHWETEIHREVHEKLQQEKNE
ZmMLO5  ----------------------------------------------------------
ZmMLO8  LLSLEAIHQLHIFIFVLAITHVIFSVTTMLLGGAQIHQWKQWENGIKKDAPGNGPKVTN-
ZmMLO9  FVSHEGLEQLHRFIFVMAVTHVTYSCLTMLLAILKIHKWRDWEDEAFRDNHESFSQIAYE
ZmMLO4  FVSYEGLEQLHRFLFILGITHVLYSFVTVVLSMIKITYSWRKWETLAGPIAAEEEKARRT-
        ----------] P----TM Helix 3----] [----Intervening Region 3----

HvMLO1  ----------FRFTHQTSFVKRHL--GLSSTPGIRWVVAFFRQFFRSVTKVDYLTLRAGF
ZmMLO1  ----------------------------------------------------------
ZmMLO2  ----------FRLTHQTSFVRQHAS-CWSKSTITLYFVSFFRQFFRSVRKTDYFTLRHGF
ZmMLO6  ----------FRFTHETSFVRQHMN-VLNKFPASFYISNFFRQFFRSVRQADYCALRHSF

ZmMLO7  GQGSSLSIVVLHREHQDEFVHKRTKGFWMKLAVASWITAFLKQFQDSVSKSDYEALRSAF
ZmMLO5  ----------------------------------------------------------
ZmMLO8  ------------VHHHEFIKKRFKGIGKDSIILSWLHSFGKQFYRSVSKSDYTTMRLGF
ZmMLO9  S----------ATRRQPALTKSYSFRSWSQNNVVMWLVCFIAQFGQSVVRADYLILRKGF
ZmMLO4  ----------KVMRRQSTFVFNNASHPWSKNKILIWMLCFLRQFKGSIIRSDYLALRLGF
        ------------------Intervening Region 3------------------
```

TO FIG. 1B.

FIG. 1A.

2/5

FROM FIG. 1A.

```
HvML01 INAHLSQNSKFDFHKYIKRSMEDDFKVVVGISLPLWGVAILTLFLDINGVGTLIWISFIP
ZmML01 ------------------------------------------------------------
ZmML02 ISAHLSPGTRFNFRKYIKRSLEDDFKTVVGISPPLWASALAVMLFNVHGWHNLFWFSAIP
ZmML06 VNVHLAPGSKFDFDKYIDRSLEDDFKVIVGISPPLWASALIFLFLNVNGWHTMLWISIMP

ZmML07 VVEHYPEKPDIDFHKYMTRAVEYEFKRVVGISWYLWLFVILFLLLNINGWHTYFWLAFLP
ZmML05 ------------------------------------------------------------
ZmML08 IMTHCPGNPKFDFHRYMVRVLEADFKKVVGISWYLWVFVVIFLLLNVNGWHTYFWIAFLP
ZmML09 IMTHNLPPT-YDFHNYMIRSMEEEFEKIVGVSGLLWGFVVAFMLFNVDGSNLYFWIAILP
ZmML04 MTYHKLPHS-YDFHKYMVRSMEDDYNGTIGISWPLWAYAIVCILINVHGINIYFWLSFVP
       ---Intervening Region 3--] [----TM Helix 4---] [IR 4] [-----TM

HvML01 LVILLCVGTKLEMIIMEMALEIQDRAS--VIKGAPVVEPSNKFFWFHRPDWVLFFIHLTL
ZmML01 -------------------EIQDKAT--VIKGAPVVEPSDRFFWFNRPGWVLFLIHLTL
ZmML02 LVVILAVGTKLQAIIAMMAIEIAERHT--VIQGMPVVKLSDDHFWFGKPRLVLHLIHFAS
ZmML06 VVIILSVGTKLQGIICRMAIDITERHA--VIQGIPMVQVSDSYFWFARPTFVLFLIHFTL

ZmML07 LFLLLIVGAKLEGIITRLAQEAAISLSN-NTEEVPKIKPCKDHFWFHKPELVLHLIHFIL
ZmML05 ------------------------------------------------------IHFIL
ZmML08 LILLLAIGTKLEHVIAQLAHDVAEKHT--AVEGDVIVKPSDEHFWFGKPRVILYLIHFIL
ZmML09 VTLVLLVGAKLQHVIATLTAEGAKMST-----YGPRIQPRDDLFWFKKPEFLLWLIHFVL
ZmML04 VELVLLVGTELQHVIAQLALEVAEATA--PY-VGSQLKLRDDLFWFGKPRVLWWLIQFIS
       Helix 5] [---------------Intervening Region 5----------------

HvML01 FQNAFQMAHFVWTVATPGLKKCYHTQIGLSIMKVVVGLALQFLCSYMTFPLYALVTQMGS
ZmML01 FQNAFQMAHFVWTLLTPDLKKCYHERLGLSIMKVAVGLVLQVLCSYITFPLYALVTQMGS
ZmML02 FQNAFEITYFFWIWYEFGLRSCFHDNFEFIIARVCLGATVQFMCSYITLPLAYALVSQMGS
ZmML06 FQNGFQIIYFLWILYEYGMDSCFNDSEEFVFARLCLGVVVQVLCSYVTLPLYALVSQMGS

ZmML07 FQNSFEISFFFWILVSEGFGSCMMERKPYVISRLVIGVIIEVECSYITLPLYAIVTHMTG
ZmML05 FQNAFEIAFFFWILTTYGFNSCIMDHVPFIVPRLVVGAIIQLLCSYSTLPLYAIVTQMGT
ZmML08 FQNAFEIAFFFWILSTYGFDSCIMGQVRFIVPRLVIGVVIQLLCSYSTLPLYAIVTQMGS
ZmML09 GQNAFELASFFWFWWQFGYDSCFIKNHLLVYCRLILGFAGQFLCSYSTLPVYALVTQMGS
ZmML04 FQNAFELATFLWSLWELSAQTCFMKHYYMVAIRLISGLLVQFWCLYYSTLPLNVIISQMGS
       -----Intervening Region 4--------] [ ------TM Helix6------] [---

HvML01 NMKRSIFDEQTSKALTNWRNTAKEKKKVR--DTDMLMAQMIGDATPSRGSS-PMPSRGSS
ZmML01 HMKKTIFEEQTAKAVMKWRKTAKDKVRQRE-AAGFLDVLTSADTTPSHSRA-TSPSRGNS
ZmML02 EMKRTIFDEQTAKALKKWHKAVVKKKHHK-------DSSHNSSETPSTDTTGPAGEAGEW
ZmML06 TMKQSIFDEQTSKALKNWRAGAKKKAPTG-------GSKHGGGGSPTAGGS-PTKADGDA

ZmML07 QIKLHGFGSRVHESVHGWIGLRKKPFSFWKIPGGDPNADSGREADVTRRVAKERSGSSRS
ZmML05 FFKKEIFDEHVQQSLLGWAQKAKKRKALRNNGNGSNGAAAG-SSHPSATARLELMRRAVA
ZmML08 CYKKEIFNEHVQQGVLGWAQKVKMKKGLK--G-----AAS--ASKDESITNADSAGPSVK
ZmML09 KYKAALIPRRIRETIHGWGKATRKKRRRR--RGDDSTVRTETSTVCSLTDDDEDFEDDDD
ZmML04 KFKKSLVSENVRESLHSWCKRVKDRSRHN--PLFSRNGTLTTRSVCSLDTTYETDHETNT
       ----------------------C-Terminus--------------------------

HvML01 ------PVHLLHKGM----------GRSDDPQSAPTSPRT------QQEARDMYPVVVAH
ZmML01 ------PVHLLHKYR----------GRSEEPQSGPASP--------GRELGDMYPVADQH
ZmML02 QRLHEVPVRHLHRYKTI-----AHVGGVRSPLSDPDYSDTDDTEPLSLQTRHLIPPAKQR
ZmML06 ------------------------------------------------------------

ZmML07 -------MPMAPADEI--------VTVDDVAVAAAAVGQGP------------------
ZmML05 -------LEEGSAGGN--------GSEASAAELHDTGPKL--------------------
ZmML08 -------IEMAKAG-----------EDVEIVGNTG----------------------
ZmML09 HHHHGPSYDTPRAGGRPPYLKIETHRQSGSGHDGPRPGGTPCFHPSGSGSGHAMLLRQAS
ZmML04 VCTLSRTASATSLDDQL-----TVVTVDDEPSCIEKDV----------------------
       ----------------------C-Terminus--------------------------
```

TO FIG. 1C.

FIG. 1B.

FROM FIG. 1B.

```
HvMLO1 PVHRLNPNDRRRSASSSALEADIPSADFSFSQG---------- 533 aa
ZmMLO1 RLHRLDP-ERMRPASSTAVNIDIADADFSFSMR---------- 224 aa
ZmMLO2 SLDTERAEVRVNVVETAAAPSDVLQDSFSFPRLLPPRHVPDK-- 565 aa
ZmMLO6 ---------------------------------------- 515 aa

ZmMLO7 ---------------------------------------- 499 aa
ZmMLO5 ---------------------------------------- 149 aa
ZmMLO8 ---------------------------------------- 492 aa
ZmMLO9 VSAPSSPSYRGGNNVTRSASMPGIAALRTTGSGTPTRVSHEEPT 469 aa
ZmMLO4 ---------------------------------------- 509 aa
       ----------------C-Terminus----------------
```

FIG. 1C.

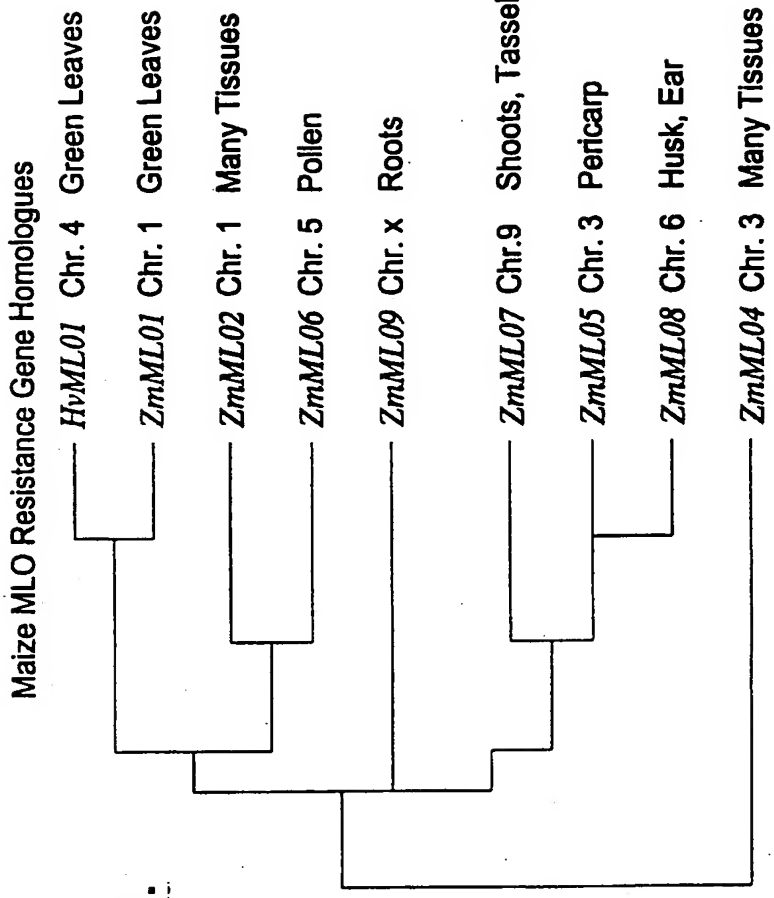
Maize MLO Resistance Gene Homologues
HvML01 Chr. 4 Green Leaves
ZmML01 Chr. 1 Green Leaves
ZmML02 Chr. 1 Many Tissues
ZmML06 Chr. 5 Pollen
ZmML09 Chr. x Roots
ZmML07 Chr.9 Shoots, Tassels
ZmML05 Chr. 3 Pericarp
ZmML08 Chr. 6 Husk, Ear
ZmML04 Chr. 3 Many Tissues

FIG. 2.

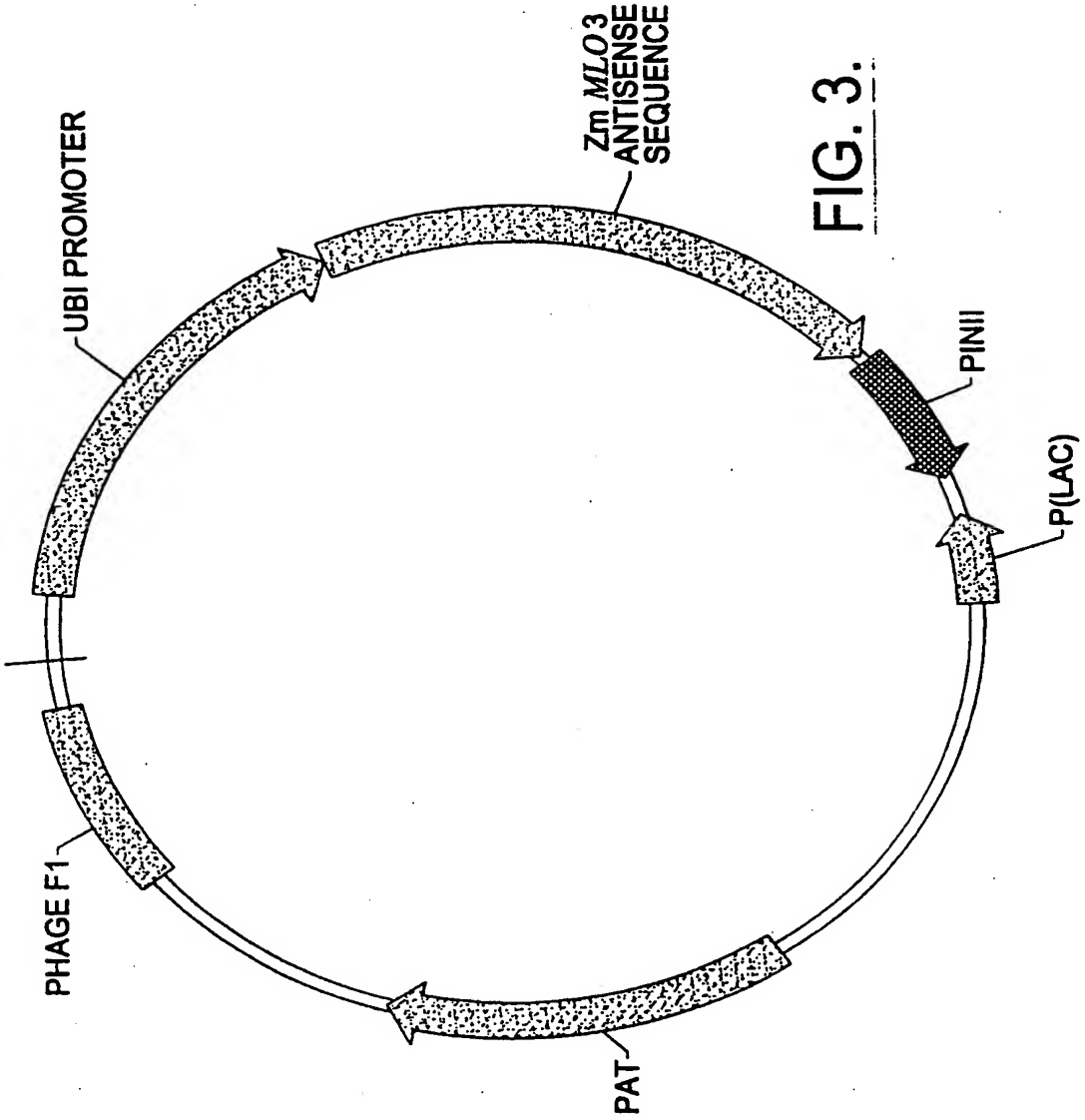
Zm MLO3 ANTISENSE SEQUENCE
UBI PROMOTER
PINII
P(LAC)
PHAGE F1
PAT

FIG. 3.

SEQUENCE LISTING

<110> Briggs, Steven

<120> Manipulation of Mlo Genes to Enhance Disease Resistance in Plants

<130> 5718-42035718/158714

<140>
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<160> 20

<170> PatentIn Ver. 2.0

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tcctctgcag ctacatcacc ttcccgctct acgcgctcgt cacgcagatg gggtcgcaca 300
tgaagaagac catcttcgag gagcagacgg ccaaggcggt gatgaagtgg cgcaagacgg 360
ccaaggataa ggtgcggcag cgggaggcgg caggcttcct cgacgtgctg acgagcgccg 420
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agcaaaagca tagacggaga acataatgac acgttacgtt agggaaagtt tcgtttattc 780
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aaaaa 845

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<212> PRT
<213> Zea mays

<220>
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<400> 2

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Leu Ile His Leu Thr Leu Phe Gln Asn Ala Phe Gln Met Ala His Phe
35 40 45

Val Trp Thr Leu Leu Thr Pro Asp Leu Lys Lys Cys Tyr His Glu Arg
50 55 60

Leu Gly Leu Ser Ile Met Lys Val Ala Val Gly Leu Val Leu Gln Val
65 70 75 80

Leu Cys Ser Tyr Ile Thr Phe Pro Leu Tyr Ala Leu Val Thr Gln Met
85 90 95

Gly Ser His Met Lys Lys Thr Ile Phe Glu Glu Gln Thr Ala Lys Ala
100 105 110

Val Met Lys Trp Arg Lys Thr Ala Lys Asp Lys Val Arg Gln Arg Glu
115 120 125

Ala Ala Gly Phe Leu Asp Val Leu Thr Ser Ala Asp Thr Thr Pro Ser
130 135 140

His Ser Arg Ala Thr Ser Pro Ser Arg Gly Asn Ser Pro Val His Leu
145 150 155 160

Leu His Lys Tyr Arg Gly Arg Ser Glu Glu Pro Gln Ser Gly Pro Ala
165 170 175

Ser Pro Gly Arg Glu Leu Gly Asp Met Tyr Pro Val Ala Asp Gln His
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Arg Leu His Arg Leu Asp Pro Glu Arg Met Arg Pro Ala Ser Ser Thr
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Ala Val Asn Ile Asp Ile Ala Asp Ala Asp Phe Ser Phe Ser Met Arg
210 215 220

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<220>
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Gly Phe Leu His His Leu Gly Gln Leu Leu Asn Lys Lys Arg Lys Lys
        35                  40                  45

Ala Leu Phe Asp Ala Leu Glu Lys Val Lys Ser Glu Leu Met Thr Leu
    50                  55                  60

Gly Phe Ile Ser Leu Leu Leu Thr Val Thr Gly Arg Tyr Ile Ala Arg
65                  70                  75                  80

Ile Cys Ile Pro Glu Gly Ala Ala Asn Thr Met Leu Pro Cys Arg Leu
                85                  90                  95

Ser Gly His Ser Val Ala Glu Glu Pro Lys Gly His Gly Arg Arg His
            100                 105                 110

Leu Ser Glu Asp Pro Thr Asn Leu Phe Ser Cys Arg Lys Gly Met Val
        115                 120                 125

Ser Leu Val Ser Ala Asp Gly Met His Gln Leu His Ile Phe Val Phe
    130                 135                 140

Phe Leu Ala Val Phe His Val Thr Phe Ser Phe Phe Thr Met Ser Leu
145                 150                 155                 160

Gly Arg Ala Lys Thr Arg Ile Trp Lys Val Trp Glu Lys Glu Thr Cys
                165                 170                 175

Ser Pro Gln Tyr Asn Tyr Leu Asn Asp Pro Ser Lys Phe Arg Leu Thr
            180                 185                 190

His Gln Thr Ser Phe Val Arg Gln His Ala Ser Cys Trp Ser Lys Ser
        195                 200                 205

Thr Ile Thr Leu Tyr Phe Val Ser Phe Phe Arg Gln Phe Phe Arg Ser
    210                 215                 220

Val Arg Lys Thr Asp Tyr Phe Thr Leu Arg His Gly Phe Ile Ser Ala
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225 230 235 240

His Leu Ser Pro Gly Thr Arg Phe Asn Phe Arg Lys Tyr Ile Lys Arg
245 250 255

Ser Leu Glu Asp Asp Phe Lys Thr Val Val Gly Ile Ser Pro Pro Leu
260 265 270

Trp Ala Ser Ala Leu Ala Val Met Leu Phe Asn Val His Gly Trp His
275 280 285

Asn Leu Phe Trp Phe Ser Ala Ile Pro Leu Val Val Ile Leu Ala Val
290 295 300

Gly Thr Lys Leu Gln Ala Ile Ile Ala Met Met Ala Ile Glu Ile Ala
305 310 315 320

Glu Arg His Thr Val Ile Gln Gly Met Pro Val Val Lys Leu Ser Asp
325 330 335

Asp His Phe Trp Phe Gly Lys Pro Arg Leu Val Leu His Leu Ile His
340 345 350

Phe Ala Ser Phe Gln Asn Ala Phe Glu Ile Thr Tyr Phe Phe Trp Ile
355 360 365

Trp Tyr Glu Phe Gly Leu Arg Ser Cys Phe His Asp Asn Phe Glu Phe
370 375 380

Ile Ile Ala Arg Val Cys Leu Gly Ala Ile Val Gln Phe Met Cys Ser
385 390 395 400

Tyr Ile Thr Leu Pro Leu Tyr Ala Leu Val Ser Gln Met Gly Ser Glu
405 410 415

Met Lys Arg Thr Ile Phe Asp Glu Gln Thr Ala Lys Ala Leu Lys Lys
420 425 430

Trp His Lys Ala Val Val Lys Lys Lys His His Lys Asp Ser Ser His
435 440 445

Asn Ser Ser Glu Thr Pro Ser Thr Asp Thr Thr Gly Pro Ala Gly Glu
450 455 460

Ala Gly Glu Trp Gln Arg Leu His Glu Val Pro Val Arg His Leu His
465 470 475 480

Arg Tyr Lys Thr Ile Ala His Val Gly Gly Val Arg Ser Pro Leu Ser

485 490 495

Asp Pro Asp Tyr Ser Asp Thr Asp Asp Thr Glu Pro Leu Ser Leu Gln
500 505 510

Thr Arg His Leu Ile Pro Pro Ala Lys Gln Arg Ser Leu Asp Thr Glu
515 520 525

Arg Ala Glu Val Arg Val Asn Val Val Glu Thr Ala Ala Ala Pro Ser
530 535 540

Asp Val Leu Gln Asp Ser Phe Ser Phe Pro Arg Leu Leu Pro Pro Arg
545 550 555 560

His Val Pro Asp Lys
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<211> 1841
<212> DNA
<213> Zea mays

<220>
<223> Mlo4

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<212> PRT
<213> Zea mays

<220>
<223> Mlo4

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Arg Ser Leu Ser Arg Phe Ala Lys Trp Leu Arg Lys Thr Lys Arg Lys
35 40 45

Ala Met Leu Ala Ala Leu Glu Lys Ile Arg Glu Glu Leu Met Leu Leu
50 55 60

Gly Val Ile Ser Leu Leu Leu Ser Gln Thr Ala Arg Phe Ile Ser Glu
65 70 75 80

Ile Cys Val Pro Ser Ser Leu Phe Thr Ser Arg Phe Tyr Ile Cys Ser
85 90 95

Glu Ser Asp Tyr Gln Asp Leu Leu Arg Asn Thr Asp Ala Asn Gln Thr
100 105 110

Ala Leu Asp Lys Asn Met Phe Gly Gly Gln Arg Leu His Val Cys Gly
115 120 125

Glu Gly His Glu Pro Phe Val Ser Tyr Glu Gly Leu Glu Gln Leu His
130 135 140

Arg Phe Leu Phe Ile Leu Gly Ile Thr His Val Leu Tyr Ser Phe Val

145 150 155 160

Thr Val Val Leu Ser Met Ile Lys Ile Tyr Ser Trp Arg Lys Trp Glu
165 170 175

Thr Leu Ala Gly Pro Ile Ala Ala Glu Glu Leu Lys Ala Arg Arg Thr
180 185 190

Lys Val Met Arg Arg Gln Ser Thr Phe Val Phe Asn Asn Ala Ser His
195 200 205

Pro Trp Ser Lys Asn Lys Ile Leu Ile Trp Met Leu Cys Phe Leu Arg
210 215 220

Gln Phe Lys Gly Ser Ile Ile Arg Ser Asp Tyr Leu Ala Leu Arg Leu
225 230 235 240

Gly Phe Val Thr Tyr His Lys Leu Pro His Ser Tyr Asp Phe His Lys
245 250 255

Tyr Met Val Arg Ser Met Glu Asp Asp Tyr Asn Gly Thr Ile Gly Ile
260 265 270

Ser Trp Pro Leu Trp Ala Tyr Ala Ile Val Cys Ile Leu Ile Asn Val
275 280 285

His Gly Ile Asn Ile Tyr Phe Trp Leu Ser Phe Val Pro Val Ile Leu
290 295 300

Val Leu Leu Val Gly Thr Glu Leu Gln His Val Ile Ala Gln Leu Ala
305 310 315 320

Leu Glu Val Ala Glu Ala Thr Ala Pro Tyr Val Gly Ser Gln Leu Lys
325 330 335

Leu Arg Asp Asp Leu Phe Trp Phe Gly Lys Pro Arg Val Leu Trp Trp
340 345 350

Leu Ile Gln Phe Ile Ser Phe Gln Asn Ala Phe Glu Leu Ala Thr Phe
355 360 365

Leu Trp Ser Leu Trp Glu Leu Ser Ala Gln Thr Cys Phe Met Lys His
370 375 380

Tyr Tyr Met Val Ala Ile Arg Leu Ile Ser Gly Leu Leu Val Gln Phe
385 390 395 400

Trp Cys Leu Tyr Ser Thr Leu Pro Leu Asn Val Ile Ile Ser Gln Met

405 410 415

Gly Ser Lys Phe Lys Lys Ser Leu Val Ser Glu Asn Val Arg Glu Ser
420 425 430

Leu His Ser Trp Cys Lys Arg Val Lys Asp Arg Ser Arg His Asn Pro
435 440 445

Leu Phe Ser Arg Asn Gly Thr Leu Thr Thr Arg Ser Val Cys Ser Leu
450 455 460

Asp Thr Thr Tyr Glu Thr Asp His Glu Thr Asn Thr Val Cys Thr Leu
465 470 475 480

Ser Arg Thr Ala Ser Ala Thr Ser Leu Asp Asp Gln Leu Thr Val Val
485 490 495

Thr Val Asp Asp Glu Pro Ser Cys Ile Glu Lys Asp Val
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<212> DNA
<213> Zea mays

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<212> PRT
<213> Zea mays

<220>
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20 25 30

Pro Phe Ile Val Pro Arg Leu Val Val Gly Ala Ile Ile Gln Leu Leu
35 40 45

Cys Ser Tyr Ser Thr Leu Pro Leu Tyr Ala Ile Val Thr Gln Met Gly
50 55 60

Thr Phe Phe Lys Lys Glu Ile Phe Asp Glu His Val Gln Gln Ser Leu
65 70 75 80

Leu Gly Trp Ala Gln Lys Ala Lys Lys Arg Lys Ala Leu Arg Asn Asn
85 90 95

Gly Asn Gly Ser Asn Gly Ala Ala Ala Gly Ser Ser His Pro Ser Ala
100 105 110

Thr Ala Arg Leu Glu Leu Met Arg Arg Ala Val Ala Leu Glu Glu Gly
115 120 125

Ser Ala Gly Gly Asn Gly Ser Glu Ala Ser Ala Ala Glu Leu His Asp
130 135 140

Thr Gly Pro Lys Leu
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<212> DNA
<213> Zea mays

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<213> Zea mays

<220>
<223> Mlo6

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Ile Leu Leu Glu Lys Gly Leu His His Val Gly Glu Phe Phe Ser His
35 40 45

Arg Lys Lys Lys Ala Met Val Glu Ala Leu Glu Lys Val Lys Ala Glu
50 55 60

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Leu Met Val Leu Gly Phe Ile Ser Leu Leu Leu Val Phe Gly Gln Asn
 65                  70                  75                  80

Tyr Ile Ile Lys Val Cys Ile Ser Asn His Ala Ala Asn Thr Met Leu
                 85                  90                  95

Pro Cys Lys Leu Glu Ala Ala Ala Val Glu Gly Lys Asp Gly His Gly
            100                 105                 110

Lys Glu Ala Ala Ala Val Val Ala Gly Lys Lys Lys Val Ala Val Ala
        115                 120                 125

Val Pro Gly Lys Lys Lys Lys Lys Ala Ala Ala Ala Ala Asp His Leu
    130                 135                 140

Gly Gly Val Val Asp Trp Pro Pro Pro Tyr Tyr Ala His Asn Ala Arg
145                 150                 155                 160

Met Leu Ala Glu Ala Ser Met Ala Thr Lys Cys Pro Glu Gly Lys Val
                165                 170                 175

Pro Leu Ile Ser Ile Asn Ala Leu His Gln Leu His Ile Phe Ile Phe
            180                 185                 190

Phe Leu Ala Val Phe His Val Ser Tyr Ser Ala Ile Thr Met Ala Leu
        195                 200                 205

Gly Arg Ala Lys Ile Arg Ala Trp Lys Glu Trp Glu Lys Glu Ala Ala
    210                 215                 220

Gly Gln Asp Tyr Glu Phe Ser His Asp Pro Thr Arg Phe Arg Phe Thr
225                 230                 235                 240

His Glu Thr Ser Phe Val Arg Gln His Met Asn Val Leu Asn Lys Phe
                245                 250                 255

Pro Ala Ser Phe Tyr Ile Ser Asn Phe Phe Arg Gln Phe Phe Arg Ser
            260                 265                 270

Val Arg Gln Ala Asp Tyr Cys Ala Leu Arg His Ser Phe Val Asn Val
        275                 280                 285

His Leu Ala Pro Gly Ser Lys Phe Asp Phe Gln Lys Tyr Ile Lys Arg
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Ser Leu Glu Asp Asp Phe Lys Val Ile Val Gly Ile Ser Pro Pro Leu
305                 310                 315                 320
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Trp Ala Ser Ala Leu Ile Phe Leu Phe Leu Asn Val Asn Gly Trp His
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Thr Met Leu Trp Ile Ser Ile Met Pro Val Val Ile Ile Leu Ser Val
340 345 350

Gly Thr Lys Leu Gln Gly Ile Ile Cys Arg Met Ala Ile Asp Ile Thr
355 360 365

Glu Arg His Ala Val Ile Gln Gly Ile Pro Met Val Gln Val Ser Asp
370 375 380

Ser Tyr Phe Trp Phe Ala Arg Pro Thr Phe Val Leu Phe Leu Ile His
385 390 395 400

Phe Thr Leu Phe Gln Asn Gly Phe Gln Ile Ile Tyr Phe Leu Trp Ile
405 410 415

Leu Tyr Glu Tyr Gly Met Asp Ser Cys Phe Asn Asp Ser Glu Glu Phe
420 425 430

Val Phe Ala Arg Leu Cys Leu Gly Val Val Val Gln Val Leu Cys Ser
435 440 445

Tyr Val Thr Leu Pro Leu Tyr Ala Leu Val Ser Gln Met Gly Ser Thr
450 455 460

Met Lys Gln Ser Ile Phe Asp Glu Gln Thr Ser Lys Ala Leu Lys Asn
465 470 475 480

Trp Arg Ala Gly Ala Lys Lys Lys Ala Pro Thr Gly Gly Ser Lys His
485 490 495

Gly Gly Gly Gly Ser Pro Thr Ala Gly Gly Ser Pro Thr Lys Ala Asp
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Gly Asp Ala
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<212> DNA
<213> Zea mays

<220>
<223> Mlo7

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Arg Thr Cys Ile Pro Glu Arg Trp Thr Phe His Met Leu Pro Cys Glu
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195 200 205

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INTERNATIONAL SEARCH REPORT

Int. onal Application No
PCT/US 99/15255

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/415 C12N15/82 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 04586 A (INNES JOHN CENTRE INNOV LTD ;PANSTRUGA RALPH (GB); BUESCHGES RAINE) 5 February 1998 (1998-02-05) the whole document ---	1-11, 13-17
X	DATABASE EMEST2 'Online! EMBL AC/ID AA660856, 14 November 1997 (1997-11-14) COVITZ P A ET AL.: "Expressed sequence tags from a root hair-enriched Medicago trunculata cDNA library" XP002119426 abstract --- -/--	13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 October 1999	03/11/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Oderwald, H

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INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/US 99/15255

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 23235 A (RAFALSKI J ANTONI ;TARAMINO GRAZIANA (US); DU PONT (US); MIAO GUO) 14 May 1999 (1999-05-14) the whole document	1-11, 13-17
P,X	DATABASE EMEST12 'Online! EMBL AC AI668283, 17 May 1999 (1999-05-17) WALBOT V: "Maize ESTs from various cDNA libraries sequenced at Stanford University" XP002119427 abstract	13
P,X	DATABASE EMEST11 'Online! EMBL AC/ID AI621523, 26 April 1999 (1999-04-26) WALBOT V: "Maize ESTs from various cDNA libraries sequenced at Stanford University" XP002119428 abstract	13
P,X	DATABASE EMEST12 'Online! EMBL AC/ID AI649550, 1 May 1999 (1999-05-01) WALBOT V: "Maize ESTs from various cDNA libraries sequenced at Stanford University" XP002119429 abstract	13
P,X	DATABASE EMBL SEQUENCE DATABASE 'Online! EMBL AC/ID AI729043, 12 June 1999 (1999-06-12) BLEWITT M ET AL.: "ESTs from developing cotton fiber" XP002119430 abstract	13
P,X	DATABASE EMBL SEQUENCE DATABASE 'Online! EMBL AC/ID AB011444, 4 July 1999 (1999-07-04) NEMOTO Y: "Isolation of novel early salt-responding genes from wheat (Triticum aestivum L.) by differential display" XP002119431 abstract	13

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1

INTERNATIONAL SEARCH REPORT

Inter- nal Application No
PCT/US 99/15255

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	DATABASE EMBL SEQUENCE DATABASE 'Online! EMBL AC/ID AI731933, 12 June 1999 (1999-06-12) BLEWITT M ET AL.: "ESTs from developing cotton fiber" XP002119432 abstract ----	13

1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int... nal Application No
PCT/US 99/15255

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9804586 A	05-02-1998	AU 3702897 A	20-02-1998
		CA 2260363 A	05-02-1998
		EP 0917536 A	26-05-1999
WO 9923235 A	14-05-1999	AU 1450199 A	24-05-1999